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PUBLIC HEALTH ASSOCIATION

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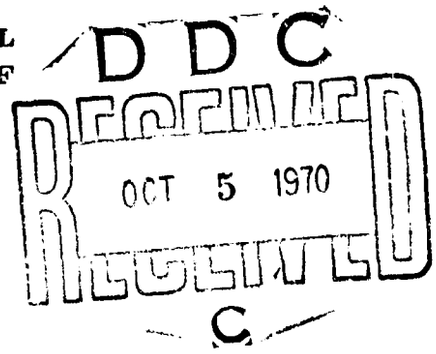
U. S. Naval Medical Research Unit No. 3

SPECIAL NUMBER

JOINT SYMPOSIUM ON TYPHOID FEVER

NAMRU-3 — ABBASSIA FEVER HOSPITAL

13 - 15 JANUARY 1970



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His Excellency The Minister of Health, Dr. ABDOU M. SALLAM (2nd from right) shown attending the scientific sessions of the joint Symposium on Typhoid held at the Conference Hall of the Egyptian Society of Political Economy, Satistics and Legislation, Cairo 13-15 January 1970.

Opening the Symposium

Ministry of Health Representative, Dr. Farag Rizk Hassan, Under Secretary of State (left) addressing the Opening Session. On his left Dr Imam Zaghloul, Chairman of the meeting and Dr. Donald C. Kent, Director, NAMRU-3.

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NAMRU-3 — Abbassia Fever Hospital

Dear Colleague

Once again I bring to you, compiled in this issue of the Journal, all the papers read in the Symposium on Typhoid which was held in Cairo on 13 - 15 January 1970.

This Symposium was organized by the staff of NAMRU-3 headed by Dr. D.C. Kent, in collaboration with the staff of Abbassia Fever Hospital headed by Dr. Anwar Hassan.

The deliberations and discussions which took place during the Symposium were a very good occasion for elucidating the lacunae in our present knowledge of certain important aspects of the disease.

No doubt that we still have well-far-to-go, in problems like immunization, prevention and eradication of the carrier state, prevention of relapses etc.

I hope that the publication of the deliberations of the Symposium will stimulate more research for better means of ridding humanity from this disease.

A.M. KAMAL, M.D.

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INTRODUCTORY SPEECH

By

Dr. DONALD C. KENT
Director, NAMRU-3

This medical symposium is devoted to the problem of typhoid fever, one of the most prevalent and serious diseases of the tropical wold. But in addition, it is a disease which attacks those areas of the world outside the tropics, oftentimes in epidemic proportion, even in those countries where the disease has been supposedly eradicated.

During the three days of presentation and discussion, the multiple disciplines necessary to study this disease in its whole will be called upon. What has been accomplished regarding the disease, what work is now on-going, and more importantly, what needs to be done will be discussed and plans made to further our quest for knowledge regarding the disease. In spite of the extremely large amount of data already accumulated, we still must expand our knowledge particularly regarding its epidemiology, control, pathophysiology, and improved treatment. We shall learn that the first effective chemotherapeutic agent, chloromycetin, remains the primary drug of choice. We must continue to search for better and less toxic

agents. We shall learn that the presently available vaccine remains less than perfect. We must develop newer concepts of prophalyxis and control. And we shall learn about the important relationship between two of the important diseases of the tropics, schistosomiasis and typhoid fever.

This symposium is dedicated to the cooperation which has existed between NAMRU-3 and the Ministry of Health and its Fever Hospitals, as well as with the entire Egyptian Medical and Scientific community. Many papers willl represent joint endeavors between these organizations in program development and research organization. To what has been accomplished with such cooperation and what is yet to develop and be carried out is the basis for this dedication. The problem of typhoid fever remains as the proverbial iceberg, much more remains unseen below the surface than already is visualized on the horizon.

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OPENING SPEECH

Dr. FARAG RIZK HASSAN
Under Secretary of State
Ministry of Public Health

Dr. Kent, Dr. Hamami, Distinguished guests,
Dear colleages, Ladies and Gentlemen.

It gives me pleasure to represent the Ministry of Public Health in this Symposium on Typhoid Fever, which is held by NAMRU-3 jointly with Abbasieh Fever hospital, here in Cairo; and to share in its activities. I welcome you all, and I sincerely hope that the presentations and discussions in these meetings may help in adding more light on the road, in an endeavor to answer some of the questions that still exist in the epidemiology of this disease.

Typhoid fever and other enteric infections are endemic under the existing environmental conditions. Under such circumstances, what are the endemo-epidemic proportions of the problem. Again, for the wide gamut of gastro-intestinal infections existing, how much of them are Typhoid and what are the others; I am thinking here of the differential diagnosis of prevailing disease-conditions. Another question is the role of carriers, and the effect on the carrier state of other existing gastro-intestinal and urinary pathological conditions e.g. Bilharziasis.

Again the laboratory diagnosis of typhoid, the clinical and epidemiologic significance and reliability of the blood culture, the Widal test, and urine and stool cultures, together with other laboratory techniques are questions worthy of discussions as regards their clinical and epidemiological significance; I am thinking here of the significance and reliability of negative findings and also of the significance and importance of these tests in epidemiologic investigations and control procedures.

These are but a few illustrations of the importance of this symposium; and, looking forwards to the results of your studies I extend to you my best wishes for the success of this symposium.

Thank you.

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TYPHOID FEVER AS AN ENDEMIC DISEASE OF IMPORTANCE IN THE MIDDLE EAST*

KENT, D.C.** and HASSAN, ANWAR***

« One man in his life plays many parts ». Such was a quotation from *Seven Ages of Man,* which well describes the diverse and varied ways in which Typhoid Fever presents and progresses (1). For these next three days we shall be discussing this subject of typhoid fever, certainly one of the most important endemic diseases of the Middle East, and the most lethal of all enteric diseases with the probable exception of acute gastroenteritis in infants. In the field of Salmonellosis great advances have been made in late years, most probably greater advances accumulated in t he last thirty years than in the previous 200 years. However in spite of this progress in our understanding, treatment and control of the disease, we still have as yet a great abyss to cross before we may relax

* Presented at NAMRU-3 Abbassia Fever Hospital Typhoid Fever Symposium, 13-15 January 1970.

** Director, NAMRU-3.

*** Director, Abbasia Fever Hospital.

and feel satisfied with our job completed. It is to lead us in seeing the way to cross this abyss that this symposium is dedicated.

Fevers which were most likely typhoid fever have been with us and recognized at least from the days of Hippocrates. Emperor Augustus of Rome certainly fell ill with the disease during his reign, and his physician, Antonius Mesa, distinguished himself by his successful treatment of his royal charge. The basis of his therapy was the application of cold baths, a treatment which remained in vague as late as during the practice and writing of the famous Sir William Osler. It remained for Willis in 1659 to outline the classic description of typhoid fever as we now know it (2). His diagnosis was entirely clinical, as was true with all of the practice of medicine in the seventeeth century, however he was undoubtedly correct with few exceptions. This is but a gentle reminder that now, as 300 years ago, our diagnosis remains essentially a clinical one, with laboratory finding playing certainly a secondary and mainly collaborative role. Willis' therapy was as described by himself « let blood, vomit, and purge ». His resultant mortality was probably not greatly variable from ours of today, even in spite of the application of effective antibiotic therapy, as first introduced by Woodward in 1948 (3)., The epidemiology of the disease was well known as early as the middle of the last century, actually even before the causative agent was discovered. For in 1853 William Budd of Devon stated that it was an alimentary infection due to infective materials in the feces contaminating water and milk and food products (4). It remained untill 1880 for Berth to discover the causative organism (5).

The disease has remained a scourge of mankind for many hundreds ofyears. During the Middle Ages it ravaged Europe in epidemics. In the Middle of the 19th century, Great Britain was being struck by up to 50,000 cases per year, and even then typhoid was acting as no respector of class, for in 1861 Prince Albert, the Prince Consort, died of the disease. It has

thus remained in many parts of the world as a serious disease with high morbidity and significant mortality.

Considerable degree of control has been evolved in many parts of the world, where the incidence has been drastically decreased in communities where health and hygiene have reached high standards of development. However even there, when hygienic standards decrease by accident, when carriers infect water or food, or during wars, famines, or droughts, when proverty and neglect create the requisite conditions, epidemics still occur. In areas where standards of education and hygiene have not as yet reached optimum conditions, governed mainly by forces of nature, significant endemic salmonellosis remains, and highly significant epidemics still occur, especially in times of flood, famine or drought.

Significant advances have been made throughout the Middle East in most countries, however endemic typhoid fever remains in most countries. Representative statistics are rarely available to signify the actual incidence as it now occurs in most countries. We hope today to be enlightened by the statistics from the UAR. The most recent figure available to this author was that of approximately 1500 cases per 100,000 population per year. Statistics from other Middle East countries are less accessible, and probably less representative of the true incidence, for variance in reporting procedures and availability of diagnostic and bacteriologic procedures result in wide divergence between the reported and true incidence. Suffice it to say that typhoid fever in the Middle East remains a highly significant endemic disease, and one of the most important of enteric infections. In most areas of the region the disease exists in wide spread distribution, however due to geographic variances, some areas appear to have spotty distribution. Most countries describe sporadic cases year round with peak periods of increased incidence, most describing one peak season, others two. The usual peak season occurs during the summer months, especially during the hot, arid months. Other countries describe a peak just

prior to the onset of the rainy season, in areas where such occur.

In all countries typhoid fever predominates, with significantly decreased incidence of paratyphoid A and B, however in most areas the exact distribution of the etiology is relatively poorly studied and not well known. Many areas of the Middle East describe higher incidence in the non-native populations, as ascribed to the high degree of immunity which is developed in the native population during early life. A similar postulate is described as the basis for the sporadicity of the infections without widespread epidemics, occurring as well mainly in children below the age of 12. In spite of this widespread immunological background, which has in some parts of the Middle East been serologically authenticated, localized outbreaks do continue to occur, due to massive contamination of water supplies, shellfish, food products, milk, eggs, etc... With rapidly developing improvements in community sanitation, the making available of piped clean, safe water supplies to all towns and villages, and general vaccination programs, the incidence of the disease has continued to diminish. In Egypt, it is now said that 90 % of all villages have clean drinking water, as compared to 11 % before the 1952 revolution, a magnificant stride forward toward the eradication of the disease. But in spite of these public health advances, we are still faced with the disease, as sporadic and endemic disease. Thus it remains up to us to continue to move forward in our prevention and control and treatment of the scourge, typhoid fever.

Few diseases offer a spectrum of interests to the multiplicity of disciplines in medicinal practice as typhoid, for few conditions exist which cannot be mimicked by it. It has appeared also of interest to the song writer; who has described the problem of the carrier as well as the English writer who wrote about Mollie Malone: «She died of a fever, and no one could save her, and that was the end of Sweet Mollie Malone. But her ghost wheels her barrow, thru streets wide and narrow, crying cockles and mussels, Alive, Alive - o».

And thus we move into this symposium, hoping to answer the plead of R. L. Huckstep (1), the last medical writer to present a volume on typhoid fever to the medical profession. For he dramatically said in 1960 : « The final stage is set but the final curtain has yet to fall. The final act has yet to be played, the final weapon to be found. Until they have been, typhoid will remain, if not as deadly and as common, at least as potentially dangerous as of old. The earlier diagnosis is of no less fundamental importance than universal prophalyxis and efficient treatment of both case and carrier, if this, one of the most lethal diseases known to man, is to be eradicated from its present position as a scourge to the East, and the potential menace to the West. »

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STATISTICS AND EPIDEMIOLOGY OF TYPHOID FEVER IN UAR

By

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By the term typhoid fever we mean statistically and epidemiologically; Typhoid, Paratyphoid A, B, and C.

1 — *Incidence of Typhoid fever in UAR:*

1-1 Statistics show that typhoid fever is an endemosporadic communicable disease in Egypt. Its incidence was steadily rising every year up to 1963. (Table-1) So the total number of cases recorded in 1950 was 7886 giving morbidity rate (m R) of 38.5 per 100.000 population. While total number of recorded cases in 1955 was 14835 giving (m R) of 64.5 per 100.000 population. Again total number of recorded cases in 1960 was 18113 giving (m R) of 69.7 per 100.000 population. The morbidity rate continued its steady rise to 74.1 in 1963.

Since 1964 (mR) of typhoid fever started to decline (Chart 1). It went down to 60.9 in 1965. The incidence of the disease

Typhoid and Paratyphoid in U.A.R.: Case Morbidity Rate per 100,000 of pop. 1950 -- 1968

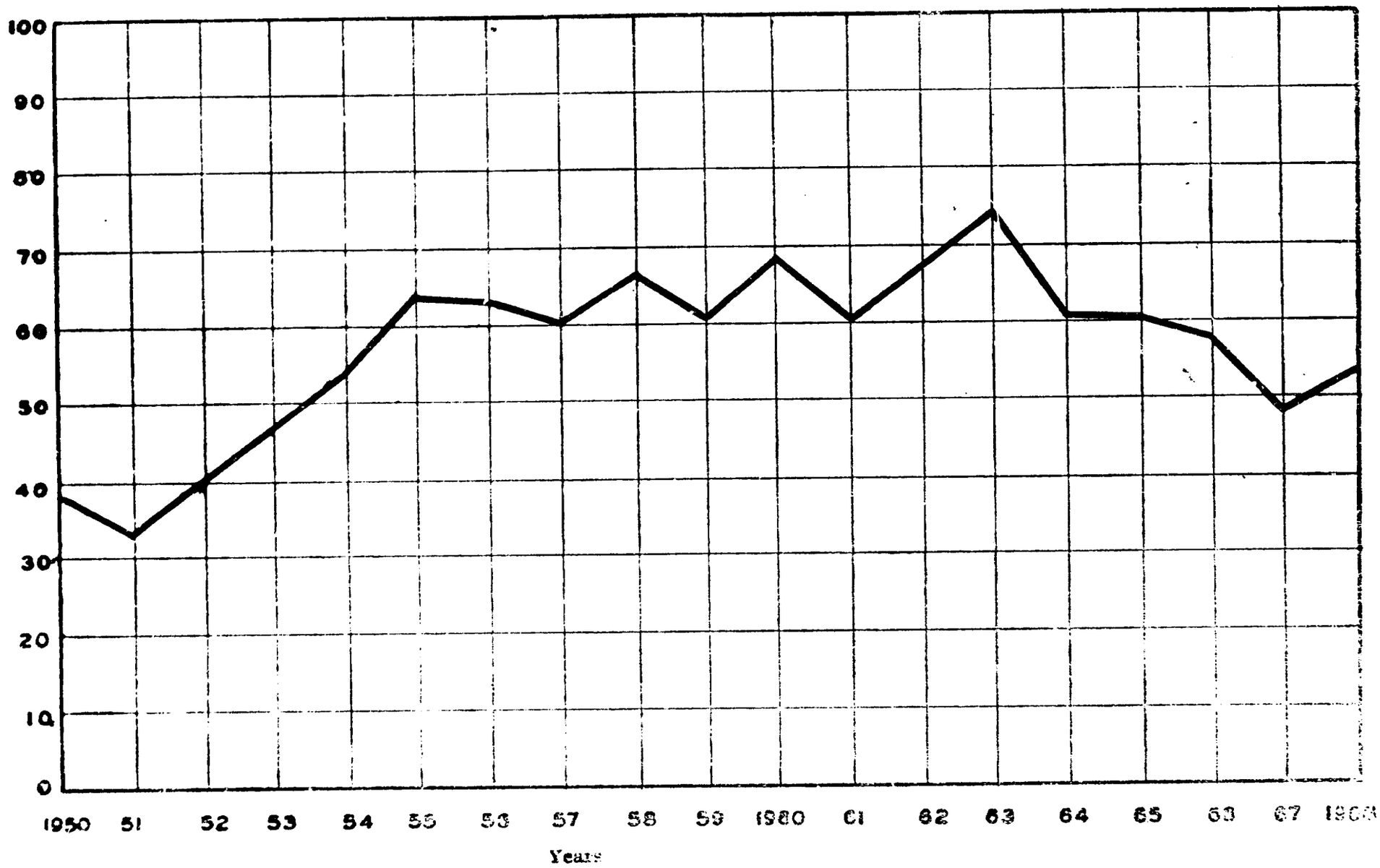


TABLE 1 — Typhoid and Paratyphoid in UAR. Cases and Case Morbidity Rate per 100,000 of population 1950-1968

Years	Cases*	M.R. per 100,000
1950	7,886	38.5
1951	6,794	32.4
1952	8,606	40.1
1953	10,156	46.3
1954	12,111	53.9
1955	14,835	64.5
1956	14,833	63.0
1957	14,339	59.5
1958	16,663	67.5
1959	15,478	61.2
1960	18,113	69.7
1961	16,307	61.4
1962	18,731	68.7
1963	20,703	74.1
1964	17,488	61.0
1965	17,902	60.9
1966	17,584	58.3
1967	15,127	48.9
1968	16,871	53.2

* These figures include typhoid and paratyphoid cases.

continued going down, so in 1968 the total number of cases recorded was 16891 making (m R) of 53.2 per 100.000 population.

To my opinion the recent decline in the incidence of typhoid fever is due to the spread of basic health services which had started since that year 1962 to cover the whole country. The decline goes hand in hand with the degree of spread of the basic health units.

1-2 *Incidence of Paratyphoid:*

The total number of paratyphoid cases — as a whole — recorded during the first half of the year 1969 was found to be 748 cases distributed as follows:

Para A 535 cases

Para B 201 cases

Para C 12 cases.

This shows clearly that Para A is predominating, while Para C is rare in UAR.

The total number of typhoid and paratyphoid cases recorded during the same period was 5237. So paratyphoid cases are nearly about 14% of the recorded cases.

The distribution of Paratyphoid cases among the Governorates (Table 2) shows that in some of them which are: Port Said, Sharkeia and Garbeia, it seems that Paratyphoid B is predominating during that period.

2 — *Mortality Rate of Typhoid Fever:*

The case fatality rate in typhoid fever is completely changed since introduction of the broad spectra antibiotics. Table 3 shows that during the last five years, the mortality rate of typhoid fever is about 1% in average. It is ranging from 0.95 to 1.34.

3 — *Geographical Distribution of Typhoid Fever:*

By analysing table 1, it shows that the incidence of typhoid fever is higher in towns and cities than in villages. This is not most probably, only due to undermodification in rural areas than in urban ones. One of the explanations for this observation is the fact that in towns people share others in the same source of food, milk and water supply, while in villages usually each house has its own food and milk supply. So if any source is contaminated, infection will be limited to a very few numbered of cases.

Here I have to mention a few words about a local outbreak of typhoid fever in El-Areesh town Capital of Sena. At the beginning of December 1964 an outbreak of 87 cases typhoid fever had occured among the pupils of the schools in El-Areesh. The epidemiological survey done there showed:

TABLE 2 — Paratyphoid in UAR. Cases recorded during the Period 1st January up to the end of June 1969 Distributed Among Governorates.

Governorate	Cases		
	Para A	Para B	Para C
Cairo	227	79	9
Alex.	136	22	3
Port Said	2	6	—
Suez	—	—	—
Ismaillia	—	1	—
Damietta	17	6	—
Behera	3	—	—
Gharbia	3	7	—
Kafr el-Sh.	3	—	—
Menupheia	2	2	—
Dakahlia	21	7	—
Sharkia	7	10	—
Qualyubia	19	6	—
Giza	6	3	—
Fayum	24	7	—
Benisuef	15	15	—
Minya	13	3	—
Asyut	12	8	—
Sohag	25	20	—
Qena	—	—	—
Aswan	—	1	—
Wadielgedid	—	—	—
Matruh	—	—	—
Sina	—	—	—
Bahr el-Ahmar	—	—	—
Total	535	201	12

TABLE 3 - Typhoid and Paratyphoid, Recorded cases and Deaths in UAR Years 1965 — 1968 (Mortality Rate %)

1965			1966			1967			1968		
C	D	%	C	D	%	C	D	%	C	D	%
17,962	240	1.34	17,384	170	0.96	15,127	161	1.06	16,871	171	1.01

TABLE 4 — Recorded cases of Typhoid and Paratyphoid during 1963-1968 distributed among Governorates

Governorate	1963	1964	1965	1966	1967	1968
Cairo	10,584	9,424	9,170	8,384	7,132	8,165
Alex.	1,615	1,247	1,704	2,504	1,564	3,037
Port Said	138	140	168	205	160	223
Suez	532	362	347	354	136	42
Ismailia	21	31	321	293	177	78
Damietta	269	142	200	381	466	525
Behera	349	200	150	339	410	442
Gharbia	1,204	743	651	850	772	534
Kafr-el-Sh.	187	212	219	203	128	61
Menufia	323	212	271	303	449	253
Dakahlia	267	324	344	412	320	125
Sharkia	93	66	95	98	89	509
Qualyubia	810	503	819	237	356	397
Giza	2,152	2,121	1,276	1,399	1,053	730
Fayum	705	552	554	149	620	418
Benisuef	73	68	89	82	82	35
Minya	309	181	301	301	366	510
Asyut	289	117	146	204	131	155
Sohag	542	553	776	571	557	533
Qena	133	144	168	183	75	41
Aswan	76	106	112	104	58	49
Wadi-el-gedid	1	—	2	15	18	1
Matruh	13	6	6	7	7	5
Sina	17	33	13	6	—	—
Bahr el-Ahmar	1	1	—	--	1	3
Total	20,703	17,488	17,902	17,584	15,127	16,871

a — All the cases were within the school age except one case was an adult of 28 years and he was a teacher in one of the schools affected.

b — During that time the schools were served a diet consisting of bread, cooked beans (Medammes) and sweet (Halawa Tebenea). On 14th and 16th of November 1964 the sweet was replaced by fresh dates, which had been washed by pouring a sac full of water from a small local pool just before delivery.

c — The cases appeared only in the schools which had received dates while not a single case had occured in the schools which did not receive dates (Rafah and Beer El-Abd.)

d — Now it is clear that the epidemiological picture of the outbreak does not belong to a water borne infection, but it is due to consuming contaminated dates by the pupils affected.

e — Serological tests of blood samples taken from 16 cases gave positive results (Widal test) for Para A giving rising titre from 1/250 up to 1/1600. By the way, by re-asking the teatcher, he confessed that he had eaten dates.

TABLE 5 — Typhoid and Paratyphoid in UAR., Monthly distribution of Cases recorded in years 1963 — 1968

Months	1963	1964	1965	1966	1967	1968
January	723	414	474	391	565	416
February	481	536	675	413	767	419
March	858	677	911	400	529	429
April	913	678	861	742	810	536
May	1,180	1,403	1,920	1,375	1,024	1,223
June	2,566	1,731	1,948	2,107	1,231	2,623
July	2,633	2,091	2,824	2,794	2,401	2,208
August	3,855	3,115	2,607	2,286	2,069	3,163
September	2,610	2,104	2,179	2,088	2,638	1,958
October	2,059	2,376	1,895	2,494	1,608	1,482
November	1,868	1,367	947	1,463	879	1,520
December	957	996	661	1,121	606	894
Total	20,703	17,488	17,902	17,584	15,127	16,871

4 — *Seasonal Incidence:*

Typhoid and paratyphoid could occur all the year round, but the disease runs a yearly seasonal rise during months May, June, July, August, September, October and November (Table 2). The seasonal wave starts rising in May, it reaches its peak in August then it declines again. In years where summer months are pro-

TABLE 6 — Typhoid and Paratyphoid in U.A.R, Age and Sex Distribution of cases recorded in years 1964 — 1968.

Age	1964		1965		1966		1967		1968	
	M	F	M	F	M	F	M	F	M	F
0—	2055	1485	1896	1544	1467	1302	1423	1050	1568	1153
5—	3840	2804	3975	2823	3752	2696	3452	2719	3683	2445
15—	3625	2169	3636	2407	3496	1996	3060	2068	4025	2356
45—	802	465	809	664	1418	1062	729	557	1010	500
65+	151	84	80	54	206	185	35	24	88	48
Unknown	4	4	9	5	2	2	9	1	—	1
Total	10477	7011	10405	7497	10341	7243	8708	6419	10374	6497

longed a 2nd wave of rise is expected in October as had happened in 1964 and 1966 (chart 2).

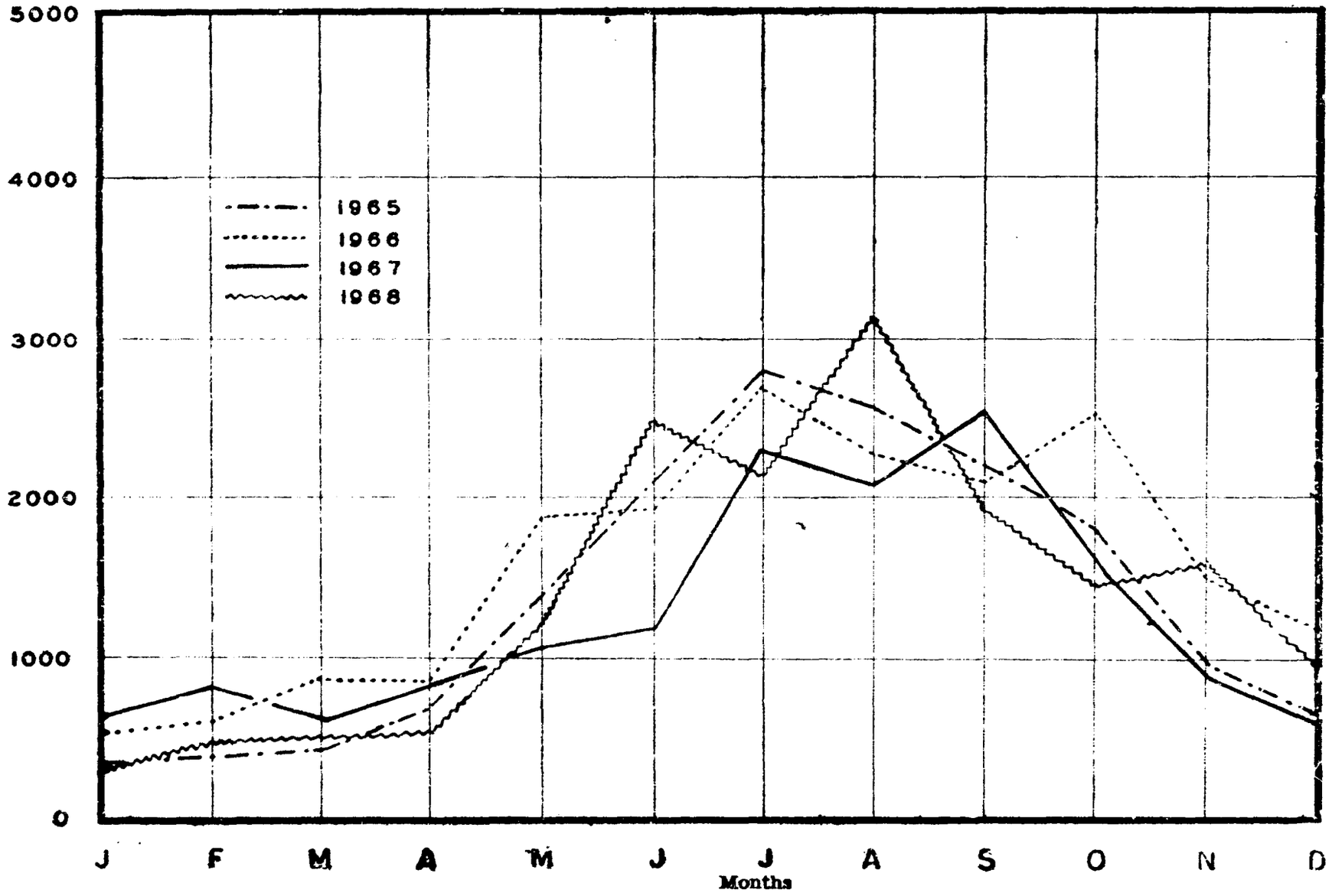
5 — *Age and Sex Incidence:*

5-1 As table 6 shows, all ages are susceptible to infection with typhoid fever. The younger ages — age groups 5-45 are more attaked than older ages. This is expected as being more consumers of food stuffs.

5-2 As regards sex incidence: the figures show that males are more affected than females. So during 1964 out of the 17488 cases recorded, 10477 cases were males and 7011 cases were females. In 1965 out of 17902 cases, males were10405 and females were 7497. The same with 1966, males affected were 10341 and females were 7243 i.e. we can say that the rate of males to females is 3:2.

As regards carries and carrier rates, being an important item it will be the subject of another lecture.

Typhoid and Paratyphoid in U.A.R., Monthly Distribution of cases recorded in years 1965 — 1966



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CHANGING PATTERN OF ENTERIC FEVERS IN U.A.R.

by

WILSON BOCTOR*
ANWAR HASSAN**
MOHAMMED FATHY ABDEL WAHAB***

4000 to 8000 patients are diagnosed as Enteric Fever every year in each of the two Fever Hospitals in Cairo, namely Abbassia and Embaba Fever Hospitals. Most of these cases are diagnosed on clinical grounds as well as serologic tests; and few are diagnosed by blood culture as well. The classical picture of enteric fever before chloramphenicol era which started in 1948 has changed. The aim of this talk is to study the changing face of enteric fever in U.A.R. in the last 20 years.

Now I shall attempt a comparison between the present picture of enteric fevers in Egypt and the picture 20 years ago-among Egyptians. The best available reference for the picture 20 years ago, is the report of Dr. Hamli in 1950, 2 years after C.A.F. had been introduced.

This study deals with the 360 positive blood culture typhoid and paratyphoid A cases in Abbassia and Embaba fever hospitals.

Cases of Para B (Of which one had a positive blood culture) are not included in this report.

* Deputy director Embaba Fever Hospital.
** Director Abbassia Fever Hospital.
*** Staff physician Abbassia Fever Hospital.

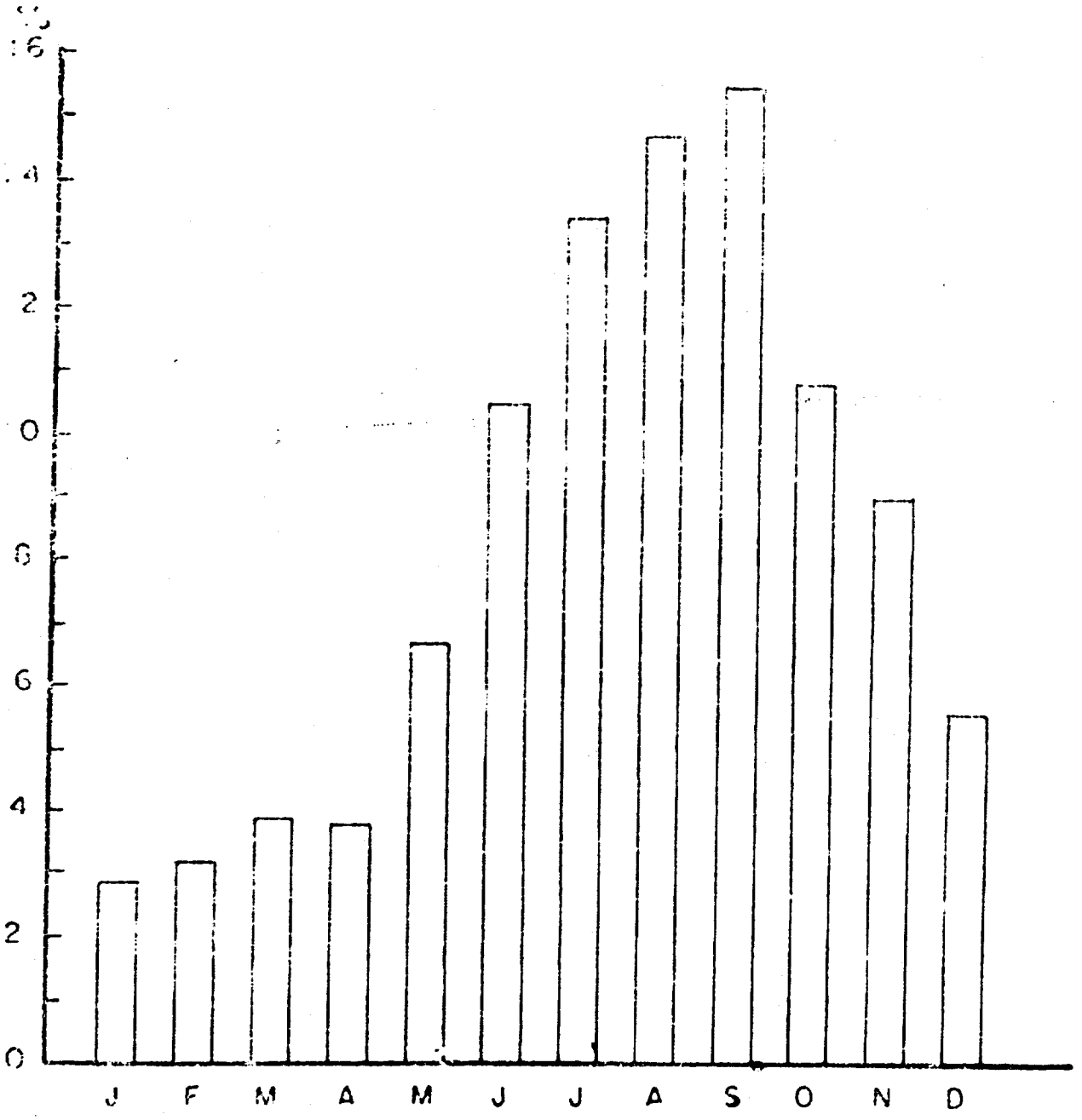

Figure 1: The monthly incidence of cases in percentage

Seasonal Incidence (Fig. 1).

Enteric fever is more prevalent in summer months, the lowest incidence is the first 3 months of the year with gradual rise until it reaches its peak in July, August, and September. This favours Kamal's (1958) findings. This seasonal incidence is known since a very long time and it has not changed.

Causative organism : Table I

TABLE I — Causative Organism

	Typhoid	Para A
No. of Cases	226	74
Percent	75	25

Two hundred and twenty six cases (75%) were due to typhoid bacillus and 74 cases (25%) due to paratyphoid A bacillus, agreeing with that of Kamal (1958).

As regards sex distribution Table II, 171 (57%) were males and the rest 129 (43%) females.

TABLE 2 — Sex Distribution

	Male	Female
No. of Cases	171	129
Percent	57	43

Age distribution: Table III

About half of the patients were below 15 years favouring El-Ramli's findings (1950).

One hundred fourty seven cases (49%) were admitted

TABLE 3 — Age Distribution

	<5	5-15	15-40	>40
No. of Cases	33	120	111	36
Percent	11	40	37	12

to the hospital within the first week of the disease, 120 cases (40%) between 7-14 days and the rest 33 (11%) after more than 2 weeks.

Two hundred and sixteen cases (72%) gave history of receiving sulphadiazine and or penicillin few days before admission. 36 patients (12%) had received a broad spectrum antibiotic in the form of tetracycline or chloramphenicol 1-3 days prior to admission. The positivity of blood culture in spite of chloramphenicol treatment agrees with Christie (1969), and Watson (1955) in South Africa.

59 patients (20%) gave positive history of bilharziasis.

TABLE 4 — Symptomatology

Symptom	No. of Cases	Percent	Symptom	No. of Cases	Percent
Chills	87	29	Cough	195	65
Headache	225	75	Epistaxis	32	11
Anorexia	255	85	Aches	27	9
Nausea and Vomiting	57	19	Diarrhea	41	14
Abd. Discomfort	269	90	Constipation	48	16
Sore Throat	78	26			

SYMPTOMS

Two hundred and sixty four patients (88%) had an insidious type of onset. The rest of the patients 36 (12%) had a

sudden type of onset. Huckstep's (1962) (Kenya and Uganda) reported cases of sudden onset of 3% only.

Chills occurred in 87 cases (29%), headache in 225 cases (75%), anorexia was noticed in 255 patients (85%). Nausea and vomiting in 57 cases (19%), abdominal discomfort in 269 patients (90%), sore throat in 78 cases (26%).

Dry cough occurred in 195 cases (65%), epistaxis in 32 cases (11%), generalized body aches in 27 cases (9%). Diarrhea was observed in 41 patients (14%) and constipation in 48 cases (16%). No herpes labialis was observed.

Atypical presentation was found in 39 cases (13%) in the form of gastroenteritis, tonsilitis, bronchitis, pneumonia and jaundice. This atypical presentation will be discussed in detail by Dr. Hathout.

Severe cases were encountered in 24 patients (8%) of our series.

The severe cases in El Ramli series were 3.5% only.

SIGNS TABLE V

Type of Temperature Charts:—

Thirty three cases (11%) had a continuous type of fever; 237 (79%) had a remittent fever, 21 cases (7%) had an intermittent temperature and in 9 cases (3%) the fever was low grade. Fever attained normal level by lysis in 267 cases (89%) and by crisis in 33 cases (11%) under chloramphenicol therapy. Relative bradycardia at the onset was present in only 86 patients (29%).

TABLE 5 — Type of Temperature and Mode of Drop of Temperature

	Conti. nous	Remit. tent	Inter. mittent	Low grade	Drop	
					Lysis	Crisis
No. of Cases	33	237	21	9	267	33
Percent	11	79	7	3	89	11

Table VI. Coated tongue was present in 216 cases (72%). Tonsilitis in 33 cases (11%), bronchitis in 92 cases (31%); of these 92 cases, 32 (11%) had wheezing rhonchi denoting asthmatic elements.

TABLE 6 — Signs

Sign	No. of Cases	Percent
Coating of Tongue	216	72
Inflamed Throat	33	11
Bronchitis	92	31
Tympanites	215	72
Abd. Tenderness	65	22
Palpabe Spleen	221	74
Enlarged Liver	87	29
Rash	12	4

Tympanitis in 215 cases (72%). Abdominal tenderness in 65 patients (22%). Felt spleen in 221 cases (74%), and enlarged liver was detected in 87 patients (29%), (we must put in consideration the bilharzial cases) and lastly rash in the form of rose spots was detected in 12 patients (4%) of our series.

Comparing these symptoms and signs nowadays with the report of El-Ramli (1950) and also of Marmion (1952) who reported on enteric fever among the British troops in the Suez Canal zone at that time, we can demonstrate the following change of pattern in enteric fever:

Epitaxis (11%) in our series is much less (Marmion 10 - 25%). Non productive irritant cough amounting to 65% of our cases (El-Ramli 50%), a fact which increases the pitfall in diagnosis of enteric fever. Sore throat (26%) also, has increased more than before.

El Ramli (1950) described stools of enteric cases as generally offensive and alkaline, may be yellowish, green and liquid (pea soup). We seldom see this picture nowadays, 70% of our patients had normal bowel habits with normal odour. 14% had diarrhea and 16% had constipation.

Intermittent type of temperature (7%) was found mostly in paratyphoid A patients with bilharzial infestation. This agrees with Hathout's (1967) and contradicts the statement of Manson (1960) who stated that a fever reaching normal level at any time of the day, is not enterica. Low grade type of fever (37.5-38°C) was found in 3% of our cases. This especially if accompanied by no specific signs or symptoms makes the diagnosis of enteric fever rather difficult. Incidence of relative bradycardia (29%) had much decreased than previously reported (Marmion 1952, 100%).

Rash in the form of rose spots (4%) has much decreased (El-Ramli 20% — Marmion 50%) and when present their number is scanty in our series.

The felt spleen (74%) has increased (El-Ramli 60%, Marmion 50%). We should remember again that some of these patients with enlarged spleen are bilharzial. Among the patients of Marmion, bilharziasis is not probable.

We could not find any significant difference in symptoms and signs between typhoid and paratyphoid A patients.

Response to Treatment:

Patients were given chloramphenicol 50 mgm/kg/body weight daily as an average, until the fever dropped to normal level. In half of the cases, the drug was continued by the same dosage for 2 days apyrexial. In the other half, the drug was continued for 7 days after normalization of temperature.

The average number of days after which the temperature dropped under C.A.F. was 5.5 days in our series. El-Ramli (1950) found that the temperature dropped to normal after an

average of 3.5 days, Marnion (1952) 4 days. So there is a definite delay in response to the drug nowadays. Drug Failure:- In two patients (0.7%), there was no response to treatment clinically and bacteriologically after periods of 12, 15 days treatment by chloramphenicol. Taneja (1957) in India, reported no response to chloramphenicol in 38 cases out of a total of 205 cases. Watson (1955) in South Africa found no response to chloramphenicol in 9% of his cases. In our cases, we have only 2 chloramphenicol failures (0.7%) of cases.

After treatment, headache was the first symptom to improve, it took an average of 3 days. Appetite is usually gained within 4 days. Abdominal discomfort was the last to improve taking an average of 4.5 days, Bowel disturbances took 1-2 days on the average to improve.

Non-bilharzial spleen subsided after an average of 11 days and liver after an average of 2 weeks from start of chloramphenicol,

Tympanites took an average of 3.5 days to subside.

Relapses:

In the cases that could be kept in hospital for 3 weeks after the drop of temperature by chloramphenicol, the relapse rate was 8%. These patients received CAF for 7 days apyrexia.

Relapse started in an average of 10.3 days apyrexial. Relapse rate in paratyphoid A patients was twice that of typhoid. Most of these paratyphoid A patients had bilharzial manifestation agreeing with Hathout 1967. In other cases that received CAF for 2 days apyrexia, the relapse rate was slightly higher reaching 10% — with an average of all the relapses of 9%. In Ramli (1950) series relapse rate was 27.5% and relapses occurred after an average of 15.5 days after drop of temperature by CAF.

Complications Table VII

Intestinal haemorrhage:

Four cases (1.3%) had intestinal haemorrhage 0,3,5 and 17 days of start of chloramphenicol therapy corresponding to 8th, 12th, 14th and 22nd day of start of illness. Bleeding was once in 3 cases and repeated in the fourth case. All patients were adults. Haemorrhage did not occur in children. In one case(bleeding occurred on the 8th day apyrexial. No colic or pain was observed during bleeding. Incidence of intestinal haemorrhage had decreased. El-Ramli (1950) reported 3% with 50% mortality rate. Huckstep (1962) also reported 3% with 38% mortality rate.

TABLE 7 — Complications

Complication	No. of Cases	Percent
Intest. hge.	4	1.3
Intest. perforation	2	0.7
Pneumonia	4	1.3
Meningeal irritation	2	0.7
Cholecystitis	10	3.3
Thrombo-phlebitis	10	3.3
Blood haemolysis	3	1
Relapses	27	9
Deaths	6	2

Intestinal perforation:

Two cases (0.7%) had intestinal perforation. This subject will be discussed in detail by our colleague Dr. Habib. The incidence of perforation has much decreased. El-Ramli (1950) reported 3.5%. The occurrence of normal or loose bowel habits is not against the diagnosis of perforation. Perforation may occur in mild enteric cases or in convalescence after the drop of temperature.

Pneumonia:

Four cases (1.3%) have been complicated with pneumonia. 3 were children and the last a 50 years old. The incidence of pneumonia had much drecreased. El-Ramli (1950) reported figure of 6% while Huckstep (1962) reported 2.8%.

Meningeal irritation:

Meningism was found in 2 children (0.7%). They had high fever, neck retraction and photophobia and both were seen at the early stage of the disease.

We had no typhoid meningitis or encephalitis. El-Ramli (1950) gave 2% incidence of typhoid encephalitis. Huckstep (1962) reported 1% incidence of typhoid meningitis.

Cholecystitis:

Ten cases (3.3%) had symptoms of cholecystitis, 7 cases occurred during the height of the fever, 3 cases during convalescence. Cholecystitis has much decreased. El-Ramli (1950) reported 7.5%. This subject will also be discussed by our colleague Dr. Habib as a surgical complication.

Thrombophlebitis

Ten cases (3.3%) has a varying degree of thrombophlebitis. Tenderness of the calf muscles was the early symptom of the disease.

Blood haemolysis:

Three patients (1%), one female and 2 males had severe haemolytic anemia that began on 4th, 5th and 8th day of chloramphenicol therapy. Patients became very pale, dyspneic, with signs of toxoemia and slight icterus. Patients experienced a sudden drop of hemoglobin and hematocrit value and increased reticulocyte (20%) count. Chloramphenicol was stopped and ampicillin, blood transfusion and steroids were given. All patients proved to have

been glucose-6-phosphate dehydrogenase deficient and/all recovered. Huckstep (1962) reported 2% incidence of severe hemolytic anemia after chloramphenicol.

Mortality Rate:

Six cases (2%) in our series died. A 23 years old male died after perforation. A 5 years old girl died on 5th admission day be complicated pneumonia. The last 4 patients 4,5-7, and 44 years old patients, were ali toxic and died on the first 3 days of admission from peripheral circulatory failure. Our fatality rate has much decreased. Kamal (1958) reported figures of 18.5% during the period 1931-1950 and 7.1% during the period 1951-1955. El-Ramli (1950) gave figure of 6.5%.

In conclusion, the picture of Enteric fever has much changed; complications are becoming less frequent and death rate is becoming much less than before. Much credit goes to chloramphenicol. We hope that in the near future, other antibiotics, more effective, less toxic, may be available for general use.

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THE PATHOLOGY OF SALMONELLOSES -- A REVIEW

By

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This review of the pathologic anatomy of salmonellosis affords a point of departure for the discussions of pathogenesis, clinical manifestations and therapeutic responses which are the focus of this symposium. The review collates the anatomic alterations of acute *Salmonella* gastroenteritis, enteric (Typhoid) fever and chronic salmonellosis, and the pathologic differential diagnosis of these conditions.

Acute Salmonella Gastroenteritis (1, 4, 7, 8, 10, 11) is most often caused by *Salmonellae* other than *S. typhosa*. It presents an acute gastrointestinal disorder 12-48 hours after ingestion of the organisms. it is rarely fatal. In experimental animals, large numbers of organisms accumulate in the lumen of the small intestine, especially the ileum (8). Such concentrations of organisms may result from either a large infective dose or when peristalsis is retarded giving the bacilli time to proliferate. Observations on humans have come from autopsies on persons who died traumatically and who had incidental infections or from surgery on patients who presented with an « acute abdomen ».

In humans, the small bowel, especially the ileum, is most frequently effected, but the stomach and colon may be effected in severe or atypical cases. The small intestine is often dilated. The serosa is erythematous and fibrinous exudate may be present in severe cases. The mucosa is erythematous with small superficial ulcerations. The lumen contains muco-purulent exudate, gas or bilious liquid chyme. The lamina propria and submucosa are edematous and hyperemic. Peyer's patches may be hypertrophic. Microscopically, the lamina propria is congested and edematous resulting in swollen villi and is infiltrated by polymorphonuclear leukocytes omit and histiocytes. Superficial ulcerations contain necrotic debris, extravasated blood, and neutrophiles. In experimental animals, numerous *Salmonellae* are demonstrated in the lamina propria and submucosa by immunofluorescent staining. Mesenteric lymph nodes are enlarged, erythematous and soft. Microscopically, the lymph nodes are hyperemic and have variable degrees of acute lymphadenitis and sinusoidal hyperplasia. In experimental animals, immunofluorescent staining demonstrates that *Salmonellae* appear first in the marginal sinuses of the lymph nodes then later spread diffusely as acute lymphadenitis develops. The spleen is mildly enlarged, soft and congested. Microscopically, the splenic red pulp is congested with slight increase in histiocytes and polymorphonuclear leukocytes.

In summary, the pattern is that of acute « catarrhal » enteritis (10), acute mesenteric lymphadenitis and acute (« septic ») splenitis. This non-specific pattern may show more histiocytes than might be expected (11), but this is not sufficient to specifically diagnose a Salmonellosis. Diagnosis is certain only after immunofluorescent staining and/or bacterial culture.

Enteric or Typhoid Fever (1, 4, 5, 7, 9, 10, 11) is most often caused by *Salmonella typhosa* but the clinical syndrome and pathologic changes occur with infection by other *Salmonellae*, especially the paratyphoid group. Though *S. typhosa* produces severe disease most frequently, paratyphoid bacilli occasionally

produce as severe a disease (11). The clinical manifestations and pathognesis of this syndrome are analyzed in detail in other papers of this symposium. Clinically, the course of the disease progresses through 5 stages : the Period of Incubation, Stage of Active Invasion, Fastigium, Stage of Lysis, and finally the Stage of Convalescence (7). The period of incubation varies in duration while the latter four stages roughly correspond to the weeks following the clinical onset. The pathologic changes approximate these stages but exact clinicopathologic correlation is not always found (4). Thus the pathologic anatomy will be discussed by stage, assuming exact clinicopathologic correlation.

1) *Period of Incubation* — Following ingestion, the bacilli proliferate rapidly in the second part of the duodenum where the bile affords an excellent culture medium. They pass through the intestinal mucosa and produce a mild inflammatory reaction comprized of histiocytes, plasma cells, lymphocytes, and a few polymorphonuclear cells and which may be diffuse or granulomatous. The bacilli multiply both intracellularly and extracellularly in the lymphoid tissues of the intestine and invade venules and lymphangioles. From the venules, bacilli enter the portal veins and go to the liver where the von Kupffer cells phagocytose them. They are then passed via hepatic lymphangioles into thoracic duct. Bacilli enter the lymphangioles of the intestinal mucosa, pass through the mesenteric lymph nodes, where they produce medullary (sinusoidal) hyperplasia and a mild lymphadenitis, and via the abdominal lymphangioles enter the thoracic duct. The thoracic duct drains into the superior vena cava, resulting in intermittent bacteremia. Thus, the bacilli become disseminated throughout the body, and become concentrated in the reticuloendothelial system (the spleen, liver, lymph nodes and bone marrow). In the liver they proliferate rapidly in the bile and reenter the intestine to begin the cycle again.

In summary, the anatomic alterations during the period incubation consist of a mild inflammation of the lamina propria of the small intestine with mild mesenteric lymph node hyper-

plasia and lymphandenitis. These inflammatory infiltrates do contain neutrophiles but other cells (histiocytes, plasma cells and lymphocytes) predominate.

2) *Period of Active Invasion.* This phase may result from the onset of continuous bacteremia. There are two types of lesions: local inflammatory lesions caused by the bacilli and multisystemic lesions caused by *Salmonella* endotoxins. The endotoxemia is greatest during the Fastigium and these lesions will be discussed in that section, but it is important to remember that endotoxin effcts commence at the clinical onset. The local reactions in this stage are most prominent in the reticuloendothelial system.

Macroscopically, the small intestine is dilated, light red, mildly injected and rarely has a serosal fibrinous exudate. The mucosa is reddened and edematous. The Peyer's patches (intestinal lymphoid aggregates which are most prevalent on the antimesenteric side of the ileum) are enlarged and raised 0.1 to 0.4 cm above the adjacent mucosa. They are oval with the long axis parallel to the axis of the intestine and may be up to 6.0 cm long. The luminal surfaces of these patches are convoluted and the margins discrete. They are soft and are slightly more erythematous than the adjacent mucosa. There is edema of the adjacent mucosa and submucosa. The cut surface of the Peyer's patch is pink to red. These changes in lymphoid tissue are most prominent in the terminal ileum but the stomach, jejunum, colon or rectum may have hyperplastic lymphoid tissue. Paratyphoid B particularly produces lesions in the stomach and rectum (4). Microscopically, intestinal lymphoid tissues are hyperplastic, hyperemic, edematous and prominently infiltrated by histiocytes (typhoid or Mallory's cells), plasma cells and lymphocytes; polymorphonuclear leukocytes are rarely observed. The histiocytes predominate in the infiltrate and actively phagocytose bacilli, erythrocytes and degenerating lymphocytes.

The mesenteric lymph nodes are markedly enlarged, soft

and pink to red. Microscopically, they have the same pattern as intestinal lymphoid tissues.

The spleen is moderately enlarged, soft and congested. Its serosal surface may have some fibrinous exudate. Microscopically, the red pulp is congested and has « typhoid nodules» which are aggregates of histiocytes (typhoid cells) similar to those infiltrating the lymphoid tissue of the intestine and mesenteric lymph nodes. The splenic white pulp is hyperplastic and germinal centers contain large numbers of histiocytes.

The liver is slightly enlarged with rounding of the liver edges and may be acutely congested if heart failure is severe. Microscopically typhoid nodules are usually distributed in the outer third of the lobule.

The bone marrow is grossly normal, but microscopically has typhoid nodules. Typhoid nodules may also be found in the kidney, testis and parotid gland.

In summary, the pathologic changes in the Stage of Active invasion consist of mild endotoxin lesions, hyperplasia and predominantly histiocytic infiltration of the lymphoreticular system, and « catarrhal » enteritis.

3) *Fastigium* — In this stage *Salmonellae* continue to proliferate in the tissues and dying bacilli release their endotoxins. Again, the anatomic alterations are due to both the local reactions to bacilli and systemic endotoxemia. The Fastigium is the «stage of necrosis» though ulceration begins late in this stage.

The macroscopic external appearance of the intestine is similar to that of the preceding stage but may be more marked. A catarrhal enteritis involves the intestinal mucosa. The Peyer's patches are raised as in the preceding stage but their surfaces have become shaggy, grey-tan and friable. The

mucosal surface may appear green due to imbibition of bile pigment. The margins of the Peyer's patches remain discrete, raised and hyperemic. The necrotic material extends down to the muscle layers and rarely through them.

The mesenteric lymph nodes are large and firmer than in the previous stage, but are not indurated. Areas of grey-tan, friable, necrotic material are seen on their cut surface and in severe cases may resemble caseation, but they are tanner and softer than caseous tissues. Microscopically, there is necrosis in the histiocytic infiltrate; initially, the necrosis is multifocal; later these foci become confluent and may destroy the entire lymph node.

The spleen is larger and more congested than in the previous stage. Microscopically, the « typhoid nodules » are larger and may be centrally necrotic. Enlargement and necrosis of typhoid nodules is also seen in the liver, bone marrow, kidney, testis and parotid glands.

The toxemic lesions of typhoid fever are extensive during this stage. There is non-specific cloudy swelling and fatty metamorphosis of hepatic parenchymal cells. The heart is flabby with biventricular dilation. Microscopically, there is fatty degeneration of cardiac myofibers. When cardiac failure is severe, acute hepatic congestion occurs. There is a mild interstitial pneumonitis and « ring hemorrhages » in the brain with microthrombi in capillaries. Peripheral neuritis has been described clinically but not pathologically. Zenkers degeneration of striated (voluntary) muscle may be severe and is most often found in muscles which remain active while the patient is in bed (the intercostals, the diaphragm, rectus abdominus and thighs). This degeneration may cause muscle rupture and hemorrhage which in the rectus abdominus muscle may simulate a surgically acute abdomen (4).

In summary, the anatomic changes during the Fastigium consist of endotoxin cellular damage in many organ systems

and necrosis in the histiocytic infiltrates formed in the preceding stage.

4) *The Stage of Lysis* is marked anatomically by sloughing of the necrotic material from the Peyer's patches of the ileum. This occurs at the end of the Fastigium and the beginning of the Period of Lysis. Macroscopically, the intestine is not different from the previous stage, but the sloughing of the necrotic Peyer's patches produce the characteristic oval ulcers with raised erythematous margins. At first the base of the ulcer is tan or green and shaggy due to remaining necrotic debris; later the ulcer becomes « clean » with the exposed pink muscle stained green to brown by bile pigment. Rarely, the ulcer extends through the muscularis. By the end of this stage, granulation tissue arises in the base of the ulcer and it becomes bright to deep red. The «catarrhal» enteritis of the non-ulcerated intestine begins to resolve late in this stage. Necrotic material in the lymph nodes and typhoid nodules cannot be sloughed and is slowly absorbed. Toxic changes may continue or previous damage may be repaired.

In summary, this stage is marked by sloughing of necrotic Peyer's patches, endotoxin effects, and the early stages of tissue repair.

5) *Convalescent Staye* — Macroscopically, the external appearance of the intestine reverts to normal; the previous fibrinous serositis rarely results in adhesions. The ulcer base granulates in and is re-epithelialized; scarring and distortion of the intestine rarely occur. Typhoid nodules are reabosrbed without distortion of tissue architecture. The capsule of the spleen may become fibrotic and « candy coated ». Toxic manifestations in cardiac muscle disappear and skeletal muscle usually regenerates.

Early and late *complications* occur during the disease. The early complications may be classified as toxic and inflammatory. Toxic complications include ileus, hepatic parenchymal

dysfunction, lower nephron nephrosis secondary to endotoxemia or to hypotension which accompanies heart failure, sudden death due to cardiac arrythmia (especially during anesthesia), venous thrombosis with pulmonary embolism, toxic encephalitis, rupture of degenerating muscles (mentioned above) and immune hemolytic anemia. These toxic complications become more frequent as the Fastigium progresses and less frequent during Lysis. The inflammatory complications are due to localization of the infection in atypical sites or abnormally extensive necrosis. Localization of the infection in atypical sites produces typhoid pneumonia and empyema (rarely), ulceration of larynx (rarely), typhoid meningitis, pericarditis, acute osteomyelitis, acute urinary tract infection, abcesses of buttocks and back and acute arthritis. When necrosis is more extensive than usual intestinal ulcers may penetrate the muscularis resulting in perforation of the intestine and peritonitis. Mesenteric lymph nodes may rupture producing mesenteric and retroperitoneal hemorrhage. Typhoid nodules may enlarge and coalesce to produce abcesses in the liver, spleen or kidney. The most frequent complication is gastrointestinal hemorrhage which occurs in 5-10% of cases and results from erosion of intestinal vessels adjacent Peyer's patches Hemorrhage is not related to size or number of typhoid ulcers. The inflammatory complications usually occur during the latter part of the Fastigium or early stage of Lysis.

The late complications usually occur during convalescence, but the differentiation between late complications and chronic disease is often difficult. Chronic cholecystitis and rarely cholangitis may develop; cholangitis is more common in cases where hepatic fibrosis due to schistosomiasis underlies the typhoid fever (6a). Chronic urinary tract infection with *Salmonellae* rarely occurs in individuals with normal urinary tracts but develops in patients with abnormal urinary tracts, especially when urinary bilharziasis underlies typhoid (6b. 9). *Salmonella* osteomyelitis may occur as an early complication but more frequently occurs during convalescence or even years after the

acute disease; it occurs commonly in children with sickle-cell disease (Hemoglobinopathy S) (4).

Chronic Salmonellosis (2, 4, 6b, 7, 9, 10, 11) or the carrier state consists of chronic low intensity infections of the gall bladder, urinary tract and possibly the intestine. These illnesses produce no pathologic alterations specific for *Salmonella* but present grossly and microscopically as chronic active inflammations of the tissue involved.

Pathologic differential diagnosis (1, 4, 7, 10, 11) — Intestine: Tuberculosis produces more exuberant lesions in the ileum and cecum; these enlarge circumferentially (rather than longitudinally as in typhoid) so that stenosis and obstruction occur. Intestinal tuberculosis involves the entire thickness of the bowel and produces much scarring. However, severe malnutrition may modify the pattern of abdominal tuberculosis, resulting in shallow ulcers closely resembling typhoid but usually lacking the raised margins characteristic of typhoid ulcers (11). *E.coli, Staphylococcus aureus* and fungi (*Candida, Aspergillus*) produce shallow intestinal ulcers with irregular margins which are not raised and have necrotic red or green ulcer bases; they also produce a pseudomembranous colitis (11). Amoebic and *Balantidum coli* ulcers are raised but not usually oval except when they become confluent; however, they are most frequently found in the cecum, ascending colon, sigmoid colon and rectum rather than in the small intestine. Strongyloidiasis may produce severe enteritis but this is usually most severe in the upper small intestine. Acute shigellosis produces a pseudomembranous colitis easily distinguished from *Salmonella* infections but may cause discrete ulcers in the chronic form; these chronic ulcers are pseudomembranous and are found in the colon. Cholera produces congestion and edema of the mucosa and submucosa but no mucosal ulceration (3). Enterovirus gastroenteritis and « non-specific » enteritis produces a « catarrhal » enteritis which may be distinguished from typhoid fever, but not from *Salmonella* gastroenteritis. Regional enteritis usually involves all layers

of the intestine and adhesions and stenoses are prominent. Falciparum malaria produces a hemorrhagic enteritis similar to the superficial hemorrhagic necrosis resulting from hypotensive splanchnic vasoconstriction; both are easily differentiated from salmonellosis. Peyer's patches are prominent in children, especially children with viral diseases. Of these diseases, measles can be specifically diagnosed by the pathognomonic Warthin-Finkeldy giant cells.

The mesenteric lymph node lesions due to typhoid must be differentiated from mesenteric adenitis, tuberculosis, melioidosis and lymphomata. Mesenteric adenitis is a non-specific entity with lymph node hyperplasia and mild subacute lymphadenitis. it may be confused with early stages of typhoid but rarely causes enteritis. Tuberculosis may be similar to the necrotic stage of typhoid but tuberculous nodes are hard. Typhoid lymph nodes are not confluent and matted as are lymphomata and tend to be softer; microscopic differentiation is not difficult.

The spleen of typhoid fever is grossly identical to other acute splenites. Microscopically, most other acute splenites present prominent polymorphonuclear infiltrates while the infiltrate of typhoid is predominantly histiocytic.

In the clinical diagnosis of acute diarrheal diseases, stool cytology can be used to rapidly differentiate exudative (*E. coli*, shigellosis, salmonellosis, and amoebiasis) from non-exudative (cholera, non-specific and viral enteritis) disease. In the exudative diseases, large members of inflammatory cells (neutrophiles, histocytes, etc.) are found in the stool while in non-exudative disease few inflammatory cells are seen (3).

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ANTIBIOTIC RESISTANCE FACTORS

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Bacterial resistance to antimicrobial drugs has been a well recognized clinical problem since the earliest days of the chemotherapeutic era. The mechanism of development and emergence of resistant strains was initially studied by classical genetic techniques and it was felt that the development of antibiotic resistant organisms usually resulted from a predictable process in which a single cell in a giant population of cells spontaneously underwent mutation toward resistance. If the population was then exposed to an appropriate antimicrobial active against the sensitive bacterial cells, the resistant mutant could then multiply to high titer and become the predominant organism. This phenomenon does occur, of course, and is sometimes responsible for the development of antibiotic resistant organisms in patients being treated with antimicrobial drugs. However, about a decade ago another mechanism — the phenomenon of infectious drug resistance — was discovered and has been shown to be

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Bacterial resistance to antimicrobial drugs has been a well recognized clinical problem since the earliest days of the chemotherapeutic era. The mechanism of development and emergence of resistant strains was initially studied by classical genetic techniques and it was felt that the development of antibiotic resistant organisms usually resulted from a predictable process in which a single cell in a giant population of cells spontaneously underwent mutation toward resistance. If the population was then exposed to an appropriate antimicrobial active against the sensitive bacterial cells, the resistant mutant could then multiply to high titer and become the predominant organism. This phenomenon does occur, of course, and is sometimes responsible for the development of antibiotic resistant organisms in patients being treated with antimicrobial drugs. However, about a decade ago another mechanism — the phenomenon of infectious drug resistance — was discovered and has been shown to be

a much more important determinant of resistance among certain bacteria, especially the *Enterobacteriaceae,* than mutation and selection.

The term «infectious drug resistance or transferable antimicrobial resistance» describes the extraordinary phenomenon whereby mixed cultivation of two different bacterial strains, even different bacterial species, one sensitive and the other resistant to one or multiple antimicrobials, is associated with the transfer of resistance from the resistant to the sensitive cells. This discovery that mixed cultivation of antibiotic resistant and antibiotic sensitive enteric bacteria can result in transfer of resistance to the sensitive bacterial strain has been shown to have important clinical and epidemiologic implications. The discovery resulted from careful epidemiologic investigations on the changing pattern of antibiotic resistant strains of Shigella in Japan (1,2). Bacillary dysentery has been an important infectious disease in Japan for many years and sulfonamides were effectively used in therapy of the disease after World War II when virtually all strains of Shigella were sensitive to this drug. However, within a few years the proportion of sulfonamide resistant strains of Shigella began to increase and the therapeutic effectiveness of sulfonamides began to decrease. The incidence of shigellosis reached an all time high in 1952. Streptomycin, tetracycline, and then chloramphenicol were employed in the treatment of sulfonamide resistant Shigella infections with excellent initial results but then within a few years Shigella resistant to these agents began to appear.

Although strains of Shigella resistant to single antimicrobials had been observed by clinicians during earlier years, strains resistant to multiple antimicrobials were not encountered until 1955. After this time, however, many epidemics of shigellosis throughout Japan were caused by organisms resistant to multiple drugs, for example, streptomycin, sulfadiazine, tetracycline, and chloramphenicol (1). Several unusual epidemiologic features were noted during these epidemics (1). For example, administration of a single antimicrobial to a patient with a totally sensitive Shigella strain was sometimes followed by the excretion of

an organism resistant, not only to the drug the patient was receiving, but also resistant to two or three other antimicrobials. This observation could not be explained easily by mutation since it is exceedingly unlikely that a single cell would spontaneously mutate toward resistance for four antibiotics at the same time. Another unusual feature was that in some outbreaks individual patients were observed that excreted antibiotic sensitive and multiply resistant Shigella strains of the same serologic type. Finally, in one epidemic of shigellosis, *Escherichia coli* strains from the stools of patients were found to have the same pattern of resistance to chloramphenicol, tetracycline, streptomycin, and sulfadiazine as the infecting Shigella strain.

No acceptable explanation of the development of multiply resistant strains or of these epidemiologic observations was available until 1959 when Akiba (3) in Japan suggested that multiple drug resistance might actually be transferred directly from multiple drug resistant *Escherichia coli* or other resistant enteric organisms to sensitive Shigella in the intestinal tracts of patients. In this event, even though transfer might be of low order, antibiotics could inhibit the sensitive bacterial strains thus permitting the resistant strains to emerge in large numbers. Shortly thereafter transfer of resistance was demonstrated *in vitro* by Akiba (4) and Ochiai (5) and their associates. In these experiments, a culture of a resistant *Escherichia coli* was simply mixed with an antibiotic-sensitive Shigella strain; after the mixture was incubated, even for only thirty minutes, and then plated on media which would inhibit all of the sensitive organisms, multiple drug resistant Shigella with the same resistance pattern as the donor *Escherichia coli* could be isolated. The frequency of transfer varies considerably but is almost never more than one transfer in 100 cells of a resistant strain and usually much less, in the range of 1 per 1000 or 1 per 100,000.

The tranfer of resistance during mixed cultivation of antibiotic resistant and antibiotic sensitive strains is mediated by extrachromosomal genetic information (genes) called resistance determinants or R factors which pass from one cell to another during actual cell contact, a phenomenon which can be termed

bacterial conjugation (1,2,6). Under these conditions, the cells actually undergo a mating process and the genetic information passes from one cell to another through tubular structures. The genetic information passed from one cell to another may carry resistance determinants for one, two or as many as six or eight antibiotics; the newly acquired information is incorporated into the chromosome of the recipient and immediately confers resistance to all subsequent progeny. By this mechanism, resistance to single or more often multiple antimicrobial drugs can be transferred *in vitro* among every genus of *Enterobacteriaceae* including *Escherichia coli*, Klebsiella-Aerobacter, Proteus species, Pseudomonas species, Salmonella, and Shigella and to other genera as well, including *Vibrio coma* and *Serratia marcescens*. The capacity of various bacterial species to donate and receive genetic information of this type varies markedly. The genetic information that is passed usually confers the capacity to make a substance which inactivates antimicrobial compounds (7,8); for example, penicillin resistance transferred by this means results from the production of a betalactamase; in other situations, chloramphenicol resistance results from the acquired property of acetylating chloramphenicol, and with streptomycin, adenylation of the antibiotic.

Transfer of drug resistance is not just an *in vitro* phenomenon and has been demonstrated to occur in the intestinal tracts of animals and man (1). The clinical and epidemiologic implications of transfer of antibiotic resistance factors are obvious. For example, pathogens such as Salmonella sensitive to antimicrobials might acquire resistance factors from antibiotic resistant organisms in the intestinal flora, multiply to high titer under the influence of antibiotic therapy, and subsequently spread to other persons, thereby producing outbreaks of infection caused by resistant organisms. Although transfer of resistance factors apparently occurs less readily in the intestinal tract than *in vitro*, transfer and emergence of resistant strains is accelerated by the selective pressures of chemotherapy as mentioned (1).

The significance and role of resistance transfer factors in the changing pattern of bacterial resistance to antimicrobial

drugs remains to be completely defined (9). However, there is enough information at the moment to indicate that it is important and apparently accounts for the antimicrobial resistance of approximately 70 per cent of *Escherichia coli*, Salmonella, Shigella, and Klebsiella-Aerobacter strains, approximately 20 per cent of the Proteus species, and approximately 5 per cent of the resistant Pseudomonas species.

A number of reports have described the phenomenon of transferable antimicrobial resistance in Salmonella. Let me mention first our own study done in 1966 (10). This study was concerned with 254 strains of Salmonella of many different serotypes isolated from clinical sources in the northeastern part of the United States. Of this group, 35 strains or 14 per cent were found to be markedly resistant to one or a combination of ampicillin, chloramphenicol, tetracycline, streptomycin, or sulfisoxazole. Seventy per cent of the resistant strains were capable of transferring their antimicrobial resistance to sensitive *Escherichia coli*. Thus, these and other studies (11, 12) have shown clearly that salmonella are capable of receiving resistance factors from other organisms and are capable of transmitting this resistance to to other sensitive recipients. The percentage of Salmonella strains that are resistant to one or more antibiotics varies remarkably and resistance is often to antibiotics which are not useful in the therapy of Salmonella infections. Resistance is much more common in serotypes other than *S. typhosa* or *S. paratyphosa*; *S. typhimurium* appears to lead the list. The phenomenon of transferable antimicrobial resistance has been described in Salmonella isolated in the United States, England, France, Greece, West Germany, the Netherlands, and probably exists throughout the world (9,10,13). One of the best studied instances of transferable antimicrobial resistance in Salmonella is that reported by Anderson (14) in England in 1964. At this time, *S. typhimurium* resistant to streptomycin, tetracycline, sulfonamides and ampicillin appeared in England. The organism was first detected in calves which were receiving antibiotics to enhance growth and then appeared to spread to man. Subsequently, the majority of strains of *S. typhimurium* isolated in England were found to be of the multiply resistant type. Prior

to 1964, only about 3 per cent of strains were found to be resistant to antimicrobial drugs whereas after 1964 over 50 per cent of the strains showed a resistance pattern, particularly in certain areas. This study seems to illustrate that Salmonella resistant by this mechanism may spread rapidly not only among the animal population, but also to man.

One might ask why infectious drug resistance has not been a much more prevalent problem among Salmonella serotypes throughout the world since multiply resistant bacteria capable of transfer have been present for a number of years and antimicrobial therapy is commonly employed in the animal and human population. The question cannot be answered completely. It is a fact that there has not been an explosive spread of multiply resistant Salmonella even though at the present time approximately 10-20 per cent of the *S. typhimurium* strains isolated in the United States and a somewhat higher proportion in England are resistant to ampicillin. However, very few strains are resistant to chloramphenicol and resistance is much less common among serologic types other than *S. typhimurium*. Throughout the world *S. typhosa* and the paratyphoid bacilli have continued to be strikingly sensitive to antimicrobial drugs; resistance to ampicillin and chloramphenicol among these serotypes is much less than 1 per cent. A number of negative factors must operate to inhibit the transfer of antimicrobial resistance and act against natural survival of strains with R factors (9). It is known that the transfer by direct contact is carried out with poor efficiency in the gastrointestinal tract. In addition, resistant organisms appear to be at a disadvantage in terms of survival and tend to disappear when placed among the normal flora of the intestinal tract in the absence of antimicrobial therapy.

Despite the prediction (9) that the major phase of epidemic dissemination of R factors is a past, not a future event, the phenomenon of infectious drug resistance inevitably brings up somewhat alarming implications — for example, the possibility of the spread of typhoid organisms resistant to chloramphenicol and ampicillin. *S. typhosa* is known to be a good recipient for resistance factors but, nevertheless, epidemics of typhoid fever caused

by chloramphenicol or ampicillin resistant strains have never been described (13). There have been a few isolated instances of isolation of *S. typhosa* with transferable resistance to chloramphenicol; these have been reported from India, Nigeria, Greece, and Israel (13,15). Paratyphoid bacilli with antimicrobial resistance factors have also been reported from France, Germany, and Greece (13, 16). It is worthy of note that Pappa (17) in 1968 reported that no chloramphenicol resistant strains of *S. typhosa* had ever been reported in North Africa though it was well-known that resistance factors were widely distributed among *Enterobacteriaceae* in this area. In the intances in which chloramphenicol or ampicillin resistant *S. typhosa* have been reported almost all have been in patients who have had typhoid fever, had shown a response to therapy, and were continuing to receive antimicrobial drugs; during this period the patients began to excrete resistant strains in stools (13,15). Thus, the organisms appeared to have acquired resistance only after reaching the gastrointestinal tract, presumably from other resistant members of the enteric flora. A similar sequence has been described in paratyphoid fever by Chabbert and Baudens (15). In none of these patients has there been evidence of secondary spread of resistant *S. typhosa* to other patients. Thus, it appears that *S. typhosa* is either a poor recipient of resistance factors in the intestinal tract of man, that resistant *S. typhosa* strains are poor disease producers, or that other factors negatively influence the development or spread of the resistant strains. Despite the evidence available at the moment indicating that chloramphenicol or ampicillin resistant *S. typhosa* is not emerging as a significant problem, we must continue to be alert to the possibility that an explosive spread of R factors could occur among these bacteria and put the therapy of typhoid and paratyphoid back to the pre-antimicrobial era. Every ampicillin or chloramphenicol resistant *S. typhosa* or *S. paratyphosa A*, *B*, or *C* should be carefully documented and studied for transferable resistance factors.

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ENTERICA OF CHILDHOOD

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In U.A.R. and the orient, enteric fever is endemic. Kamal (1957) stated that one of the epidemiological peculiarities of typhoid in Egypt, is that its morbidity prevalence is more among the younger ages. They are exposed to infection early and in a massive way, hence their higher morbidity. Kamal (1957) also emphasized the fact that the season of «enteritis and diarrhoea» and typhoid is the same and they both rise numerically during that season, leading to missing of typhoid among younger generations.

The aim of this work is to study the clinical picture of typhoid fever in children (age up to 15 years) in U.A.R. We will compare this clinical aspect in the last 2 years with our experience since 1947 till the present time.

MATERIAL AND METHODS

During the 2 years period 1968 and 1969, 150 children with the diagnosis of enteric fever, randomly taken at Abbassia Fever Hospital were analysed. The criterion for diagnosis was positive blood culture. In 128 patients (85%), typhoid bacillus was isolated from the blood and in 22 (15%) cases paratyphoid A bacillus was

the causative organism.* Eighty eight patients (59%) were males and 62 (41%) females. Fourty cases (27%) were under 5 years of age (Table I), 65 cases (43%) between 5-10 years and 45 cases (30%) between 10-15 years. Only 3 cases (2%) were under 2 years of age. Ninety two cases (61%) were admitted within the first week of the disease, 40 (27%) within second week and the rest 18 (12%) patients had history of illness more than 2 weeks.

TABLE 1 — Age Distribution

	—5 years	5—10 years	10—15 years
No.	40	65	45
Percent	27	43	30

In 29 patients (19%) the condition was considered severe. Severe cases presented by hyperpyrexia, vomiting, restlessness, convulsions, unconsciousness and occasionally evidence of peripheral circulatory failure.

Patients were given chloramphenicol capsules 50 mgm per kilogram body weight daily by the oral route. Suppository dose was double the oral route. Chloramphenicol palmitate syrup 75 mgm per kilogram body weight daily was given to children under 5 years age presenting by diarrhoea. Corticosteroids in a tapering 3 days period was given only to toxic cases.

TABLE 2 — Duration before admission

	—7 days	7—14 days	14 days
No.	92	40	18
Percent	61	27	12

* For some technical reasons we limited our group to salmonella typhi and paratyphi A.

RESULTS

Fourty two cases (28%) gave history of more of less sudden onset, the rest 108 (72%) patients had insidious type of onset. Fever was present in almost all cases. Thirty one cases (21%) had continuous type of fever, 114 (76%) had a remittent form and only in 5 cases (3%) the fever was intermittent in character. Relative bradycardia was seen in only 12 cases (8%). Fever dropped by lysis in 124 cases (82%) and by crises in 26 cases (18%). Lethargy was noted in most of our series.

In the group of 40 children under 5 years of age, (Figure 1) coughing was present in 32 cases (80%), vomiting and/or diarrhoea in 30 cases (75%). Coated tongue was observed in only 5 cases (12%). Tonsilitis was found in 18 cases (45%). Bronchitic signs was elicited in 24 cases (60%), enlarged spleen in 28 patients (70%). Tympanitis was only found in 11 cases (28%) and rash in the form of rose spots in only one patient (3%).

In the next age group between 5-10 years of age, coughing was present in 41 cases (63%), vomiting and or diarrhoea in 18 cases (28%). Coated tongue was found in 44 cases (67%), tonsilitis in 14 cases (22%). Signs of bronchitis was found in 28 cases (43%), enlarged spleen in 58 cases (89%), tympanitis in 53 cases (82%) and rose spots in 3 cases (5%).

In the 45 children between 10-15 years, the clinical picture simulates to a great extent that described by Boctor (1970).

Atypical presentation was found in 35 cases (23%). They were mostly children under 5 years of age. The disease presented as gastroenteritis in 15 (10%) cases, bronchitis or pneumonia in 11 (7%) patients and meningismus in 9 (6%) cases.

The average number of days after which temperature dropped to normal was 6.4 days (range 4-10 days).

As regards complications, intestinal haemorrhage was found in 1 case (0.7%). Intestinal perforation was encounte-

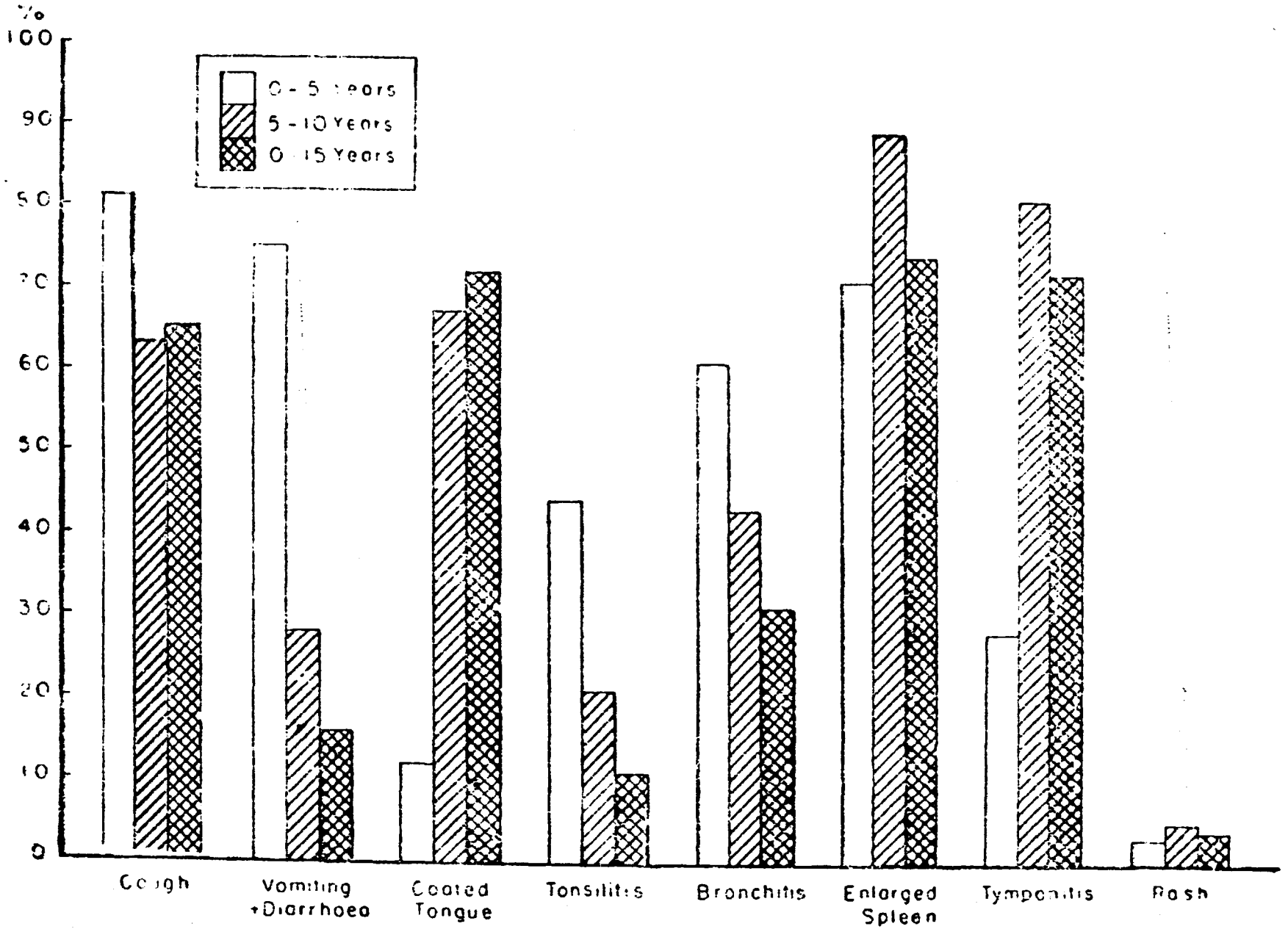

Figure 1 MAIN SYMPTOMS AND SIGNS

red also in 1 case (0.7%). Twelve typhoid cases (8%) had complicated pneumonia, deteriorating the general condition of the patients. Otitis media was present in 3 cases (2%). Seventeen cases (11%) relapsed. The average apyrexial period before the relapse was 8.5 days. Relapse were usually milder than initial attack taking an average of 5.8 days.

Ten patients (6%) died, most of them were children under 5 years of age. One case had intestinal haemorrhage, other intestinal perforation, 2 complicated pneumonia and the rest (6) succumbed early in the disease from peripheral circulatory failure.

DISCUSSION

Typhoid fever is prevalent in the younger ages in U.A.R. On the national scale, about 20% of enteric cases are below 5 years of age. Stoeckel (1935) and Trowell (1958) had encountered enteric fever in new born babies as the organisms are capable of transmission through the placenta. The widal reaction of the newborn was positive but the titer was lower than that of the mother. Blood culture was positive for enteric organisms in about half of the typhoid babies. Bacilli were not found in thier intestinal canal.

In our series, it is observed that the disease is more acute than in adults favouring those of Huckstep (1962). The fairly common sudden mode of onset (28%) was mostly observed at the younger ages. This finding coincides with that of Huckstep (1962) and Christie (1969). Abu-Ghareeb (1968) found that during the late months of summer, typhoid fever is frequently found to be vigorously attacking that many patients succumb in the first days of invasion stage before any time given for diagnosis due to toxicity and overwhelming infection. Intermittent type of fever (3%) was observed in children suffering from bilharziasis agreeing with that of Hathout (1967). Drop of fever by crisis (18%) is more than that of adults.

In the age group under 5 years of age, cough (80%), vomit-

ing and or diarrhea (75%) and tonsilitis (44%) were the predominant symptoms. Coated tongue (12%) and tympanitis (28%) were rare. This clinical picture adds to the pitfalls in the diagnosis. The general practitioner is liable to miss these enteric cases as gastro-enteritis. This is the reason for the underestimated number of cases under 5 years in our opinion in that series. We recommend transference of feverish children to fever hospitals where facilities for observation and laboratory methods are convenient and free. We have seen recently a small outbreak of vomiting and diarrhoea with fever in a small town in Dakahlia province. All the patients were children, toxic in appearance and having profuse rash resembling that of murine typhus. They proved to be due to paratyphoid *B. bacillus*. Similar findings were observed by Christie (1969). Respiratory presentation in the form of bronchitis and pneumonia adds to the pitfalls of diagnosis. Rarity of rose spots in our series is striking. During the period between 1947 — 1957, I had seen rose spots in about 10% of my enteric cases, the colour of skin of the patient, the use of good source of light and the physician's interest are important factors in spotting the rash. During our inspection rounds we confronted several cases of rashes which were difficult to collect.

It seemed that typhoid in children is milder than in adults. We should mention however that admitted enteric cases especially the age group 0-5 years, are usually severe and undernourished. Abu-Ghareeb (1968) in his critical review of post mortum in 1956 from Abbassia Fever Hospital, found that out of a total of 152 deaths during that year, 110 (72%) were children under 5 years of age. We found in year 1968 in the same hospital that about 40% (37 out of 91) of the deaths were children below 5 years.

No typhoid meningitis was encountered in our series. Turbid cerebrospinal fluid does not exclude typhoid meningitis. Culture of turbid cerebro-spinal fluid is recommended also for enteric corganisms. We have recently given established regulations that in cases of meningitis resistant to the usual therapy with Sulpha and penicillin, physicians have to examine the cerebrospinal fluid for enteric organisms to eclude it as a causative organism of meningitis.

The view that intestinal heamorrhage and perforation tend to occur less in children than in adults, agrees with that of Ramsay (1967). among the large group of patients under the age of 10 years.

As regards differential diagnosis. in the age group of up to 5 years. acute gastroenteritis and infantile malaria should be born in mind. A suckling baby was admitted with his mother suffering from enterica. Baby had severe anaemia and continuous fever. Both the mother and baby received chloramphenicol treatment. Blood picture of baby showed plasmodium malaria.

In the age group 5-15 years, acute rheumatic fever, tuberculous peritonitis, tuberculous adenitis, tuberculous meningitis, miliary tuberculosis, infectious mononucleosis, dysentry, brucellosis and lupus erythrematosis are mentioned.

SUMMARY

On hundred and fifty children with positive blood culture for typhoid or paratyphoid A bacillus were studied. In the age group under 5 years of age, enterica may present as acute gastroenteritis, bronchitis or bronchopneumonia. The disease may start at new born.

Intestinal haemorrhage and perforation were rare. The average apyrexial period before relapse was 8.5 days.

In our opinion, the clinical picture of typhoid in children has not changed much its face than that 10 years most probably due to low immunogenic status of this age group.

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UNUSUAL PRESENTATION OF ENTERIC INFECTIONS

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On 1912, Osler described the classical picture of typhoid, as an insidious febrile illness of fairly marked severity, occurring mainly and most frequently in the age group between 10 and 30 years, and usually with gastro-intestinal symptoms. Its course lasted about four weeks, and temperature reached about 104°F after a step ladder rise of one week duration, then stayed for one week, reaching the normal during the fourth week. Pulse was slow in relation to temperature, and there was an invariably enlarged spleen and rose spots. Blood culture was positive in first week, widal test in the second and stool culture in the third week of illness. Relapses when occurred were usually shorter and of milder severity than the original attack.

However, this classical pattern of enteric fever is now rarely seen, for the course of the illness is generally cut short by the use of the antibiotics. Gulati et al, 1968, demonstrated that typhoid had changed its pattern after the introduction of antibiotics. They compared their findings with those recorded by Stuart et al 1946, in their study of typhoid cases in the prechloramphenicol era. Moreover, even without the use of antibiotics

there is great variation in the severity of the illness and its clinical manifestations. This is presumably due to variable virulence and dosage of the enteric bacillus, and difference in the resistence and the immunity of the patients. The disease may be so mild to be unrecognised. Mild or abortive cases may be apyrexial throughout or may have a short course of fever. Ambulatory cases may be able to continue their work as usual, till they get cure, temperature gets high or complications occur. On the other hand, severe cases with prolonged pyrexia, marked circulatory and nervous involvement, and high incidence of complications may happen. Severe cases, with haemorrhages in bowels, kidneys, skin, mucous membranes and viscera are rarely met with. The disease may even assume a chronic form. Table(1) demonstrates the various clinical forms of the disease.

TABLE 1 — Types of Typhoid-Paratyphoid Infections

I.	Chronic form:
	1. Febrile
	2. Afebrile
II.	Acute form:
	1. Mild
	2. Abortive
	3. Ambulatory
	4. Ordinary
	5. Severe
	6. Haemorrhagic

The chronic forms of the disease:

Recently, Hathout (1960, and 1967), from Egypt and Neves (1967), from Brazil, reported that enteric infections complicating schistosomiasis usually assume a chronic form. In Egypt, we have strong evidence that enterica in patients with urinary schistosomiasis is a urinary tract, rather than an intestinal infection. Serological surveys carried out by various investigators, in the rural areas of our country, have demonstrated the high incidence of enteric infections among rural population, Miller (1950), Weir (1952), and Messih (1961). Simultaneously, there is strong evidence of the high urinary carrier rate among the same population, Miller (1954), Weir (1954) and Anwar (1954). On the other hand, it has been demonstrated that classical cases of enteric fever are rarely detected among them. Kamal (1957), stated that there is a very big difference between urban and rural incidence of typhoid, the former being 9 to 13 times as frequent as the latter, if not more. This difference is rather on the unexpected side, as with urbanisation better sanitary conditions prevail and this must affect the incidence of typhoid, not mentioning of course, the better standard of living and of education among the dewellers of urban areas. Weir et al. (1952), reported that a village called Sendibis, had been the site of a research study carried out by special staff of six physicians associated with at least 16 sanitarians and health visitors. Based on previous serological surveys, Weir expected to find 180-210 typhoid cases. However, during the period from 1948-1950, he only found two acute cases of typhoid. Therefore, it is quite evident that enteric infection is common in the rural areas of our country, but it assumes an atypical form when complicated by schistosomiasis. However, this atypical form is still not yet well recognized by the majority of our medical staff. Such patients are frequently diagnosed as cases of malaria brucella, infective hepatitis, amaebic hepatitis, or urinary tract infection secondary to urinary schistosomiasis. I can state confidently that this atypical pattern of enteric infection is the form usually encountered in schistosomal patients.

Chronic salmonellosis, include both the febrile and afebrile

form. The febrile form is manifested by prolonged hectic fever, and characterised by frequent relapses following chloramphenicol therapy, figures (1) and (2) The afebrile form include

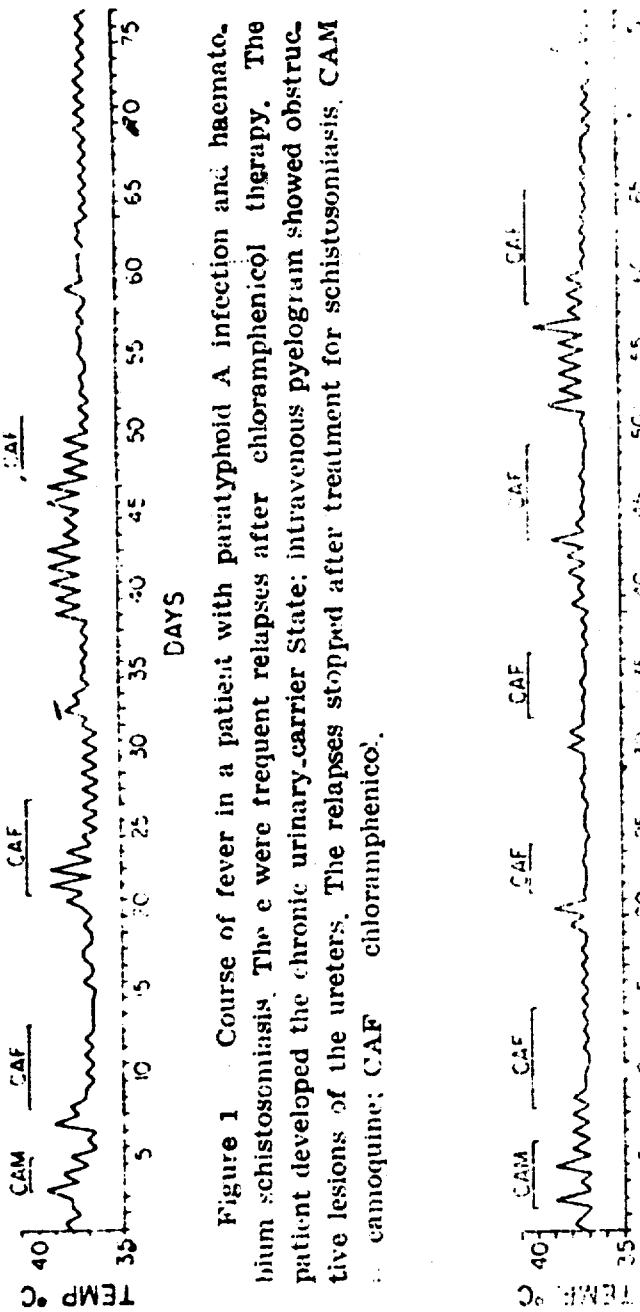

Figure 1 Course of fever in a patient with paratyphoid A infection and haematobium schistosomiasis. There were frequent relapses after chloramphenicol therapy. The patient developed the chronic urinary-carrier State; intravenous pyelogram showed obstructive lesions of the ureters. The relapses stopped after treatment for schistosomiasis. CAM = camoquine; CAF = chloramphenicol.

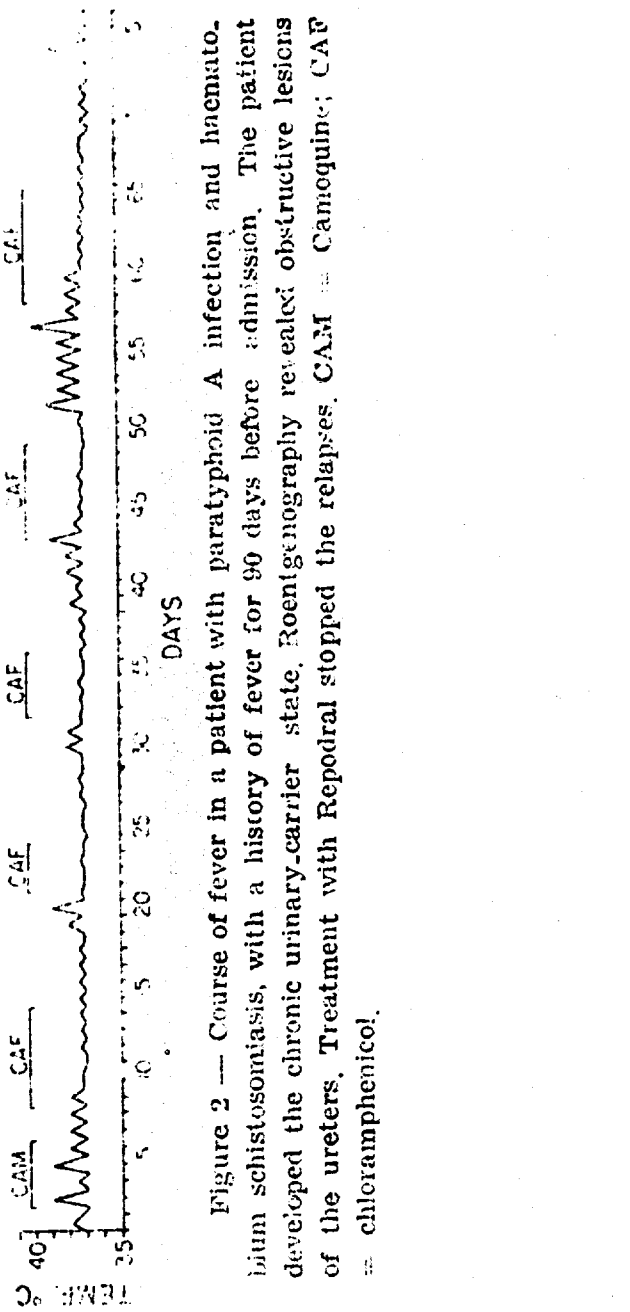

Figure 2 — Course of fever in a patient with paratyphoid A infection and haematobium schistosomiasis, with a history of fever for 90 days before admission. The patient developed the chronic urinary-carrier state. Roentgenography revealed obstructive lesions of the ureters. Treatment with Repodral stopped the relapses. CAM = Camoquine; CAF = chloramphenicol.

chronic enteric urinary carriers who do not show any significant rise of temperature, Miller (1954). Recently in NAMRU-3,

Cairo, salmonella organisms have been isolated from blood cultures of a number of urinary carriers showing no evidence of fever. Good et al. (1950), Batty et al. (1951). and Watson (1955) emphasized that S. typhi might be recovered from the blood stream in spite of the patient being apyrexial.

The following brief summary illustrates the febrile form of this chronic salmonellosis in a patient presenting with the clinical picture of amaebic hepatitis.

A farmer, 31 years old, was admitted to Ain Shams University Hospital with a history of fever for the previous eight months. He was complaining of pain in his right hypochondrium. Four months after the onset of his illness he got mild jaundice. He gave past history of schistosomal infection. On examination, he had enlarged tender liver, and he was treated as amaebic hepatitis case, but he did not show any clinical improvement. When Prof. Abdel Ghaffar, Medical Department, Faculty of Medicine, Ain Shams University Cairo, U.A.R., examined him he suspected chronic salmonellosis. Investigations revealed the results shown in table (2).

During the last two years in a series of 45 patients admitted to the Abbassia Fever Hospital as fever of unknown origin (F.U.O.) 11 per cent of the cases were proved to be of chronic salmonellosis complicated by schistosomiasis.

The unusual clinical presentations in the acute form of enteric infection:

In the old and young, typhoid fever is usually atypical and with a variable course. In children, onset is commonly acute and associated with convulsions and disease may simulate malaria, typhus, or lobar pneumonia. The child is likely to present with vomiting and diarrhea commonly resulting in dehydration, a condition resembling acute gastro-enteritis or bacillary dysentery. M. Ali from Egypt (1940), cultured the stools of 2000 children suffering from diarrhea and dysentery; 3 percent of cases were detected passing S. typhi organisms in their stools. Higgins et al. (1955), during their study on Shigellosis in the village of

TABLE 2 — Chronic salmonellosis: Febrile Form

	Case presenting as Amaebic Hepatitis Male, 31 years, farmer
Duration	: 8 months before admission
Blood culture	: - S. para A
Urine culture	: - S. para A
Widal	: PA 1/800
Urine	: Pus cells - - -, granular casts - -, R.B.C.'s - - -, albumin - +
L.C.	: 15,200 Polymorph 68, Lymph 28, Eos. 4, Mon. 0, Bas. 0.
Liver function:	
Colour index	: 11.5 units
Taymol turbidity:	: 11.5 units
Zinc turbidity	: 28 units.

Sindibis (near Cairo) were able to isolate S. typhi from the stools of 3 out of 300 children, in the age range of six months to five years. They stated that in view of limitations of bacteriological methods employed, it was possible that more Salmonella infections had been present, and had not been indentified. Floyd, (1953), as stated by Kamal (1957), doing blood cultures from 2000 cases of undiagnosed fever in children at Qalyub district, near Cairo, reported 5 per cent of cases with cultures for Salmonella organisms.

Central nervous and respiratory manifestations are also common unusual presentations among children.

Table (3) demonstrates unusual presentations and the various systems of the body which may be involved.

TABLE 3 — Unusual Clinical Presentation in the Acute Type

I.	Onset: Sudden, rigors, convulsions may simulate influenza, typhus; malaria.	
II.	Rash: profuse rash.	
III.	Presentation:	
	1. Respiratory:	Tonsilitis, otitis media, bronchitis, pneumonia, pleurisy, empyema.
	2. Gastro.intestinal:	Gastro enteritis, dysentery, cholecystitis, appendicitis, hepatitis (amoebic and viral).
	3. Muscle and joint:	muscular pains, arthritis.
	4. Cardiac:	Carditis, functional murmur.
	5. Genito.urinary:	pyelitis, nephritis, orchitis.
	6. Nervous:	meningism, encephalitis, meningitis.
	7. Osseous system:	typhoid spine, Broad's abscess.
IV.	Antibiotic therapy:	under dosage, irregular administration.
V	Complications as presenting manifestation:	Intestinal haemorrhage, perforation.

Our collegue Dr. Wilson, stated that in a combined study of 300 typhoid and paratyphoid patients admitted to the Abbassia and Embaba Fever Hospitals, during the last two years, 13% of cases showed atypical presentations including acute gastro-enteritis, tonsilitis, pneumonia, jaundice, and encephalitis. However, Dr. Kamal Mousa mentioned that in a series of 150 children with positive typhoid and paratyphoid A blood cultures admited to the Abbassia Fever Hospital during the same period of the previous study, atypical forms were noticed in 24 per cent of cases. This agrees with the general belief that enteric infection assumes an atypical form in children more than in adults. From India, Gulati et al (1968), stated that 36 per cent of typhoid cases had unusual presentations.

Onset of enteric fever may be abrupt and within a day or two the patient becomes acutely ill. Patients may complain of severe vomiting, rigors and or convulsions. Chills and rigors are, in some cases, due to administration of antipyretics and when the latter are with held, no further rigors take place ... This acute onset is usually noticed in children. In Abbassia Fever Hospital during the period 1966-1969, sudden onset of fever was recorded in 12 per cent of 300 typhoid and paratyphoid patients and most of them were children. Temperature charts showed continous fever in 11 per cent, remittent, and intermittent type, in 79 and 7 per cent respectively, low grade type of fever was recorded in 3 per cent of cases. Fever returned to normal level by crisis in 11 per cent.

Rose Spots are not so commonly observed in our patients as in the white coloured races. Only 4 per cent of 300 cases showed rose spots in the Abbasia Fever Hospital However, the rash may be profuse and may closely simultate typhus rash. This occurs more commonly in paratyphoid patients.

Few cases persent as acute tonsilits or otitis media. Both conditions are seen much more frequently in children than in adults. Last Decembre, 1 met an interesting case of a boy 15 years old, who had recurrent attacks of acute otitis media of his right ear. On the 17th of December 1969, he complained of sudden rise of temperature with pain in the right ear and on the same day he was put on tetracyclin for the acute otitis media. The high fever persisted, although the ear condition had improved as it was verified by Prof. El Mofty, Chief of E.N.T. Department and Dean of Ain Shams University Faculty of Medicine, Cairo, U.A.R. On examination the spleen was felt on the 8th day of illness. Investigations done on the same day revealed:

L.C. 6200	
Staff nucleated	5%
Staff segmented	52%

Lymphocytes	33%
Eosinophils	4%
Monocytes	5%
Basophils	1%

Widal test was negative while the blood culture was positive for Sal. paratyphi A. organisms. Patient responded quite well to chloramphenicol therapy.

A mistaken diagnosis of bronchopneumonia may be made on account of wide spread crepitations but respiratory physical signs are usually mot severe enough to account for the marked toxaemia.

Acute cholecystitis is commonly observed during and after enteric fever. Occasionally, typhoid and paratyphoid patients present with acute cholecystitis. Abu Ghareeb 1968. reported several post-mortem cases with acute typhoid ulcers in the gall bladder, during his 25 years practice in Abbassia Fever Hospital. He also recorded two cases of peritonitis secondary to perforated typhoid ulcers in the gall bladder Last year, Dr. Khalaf, the surgeon of the Abbassia Fever Hospital, operated on a paratyphoid A patient with peritonitis secondary to perforated gall bladder.

Mild jaundice may be present in some cases but sometimes it is marked and occurs early in the disease and condition closely resembles infective hepatitis. We came accross similar cases and S. typhi and S. paratyphi organisms were isolated from their blood cultures. From Nigeria, Ikeme et al. (1966) reported 18 cases with jaundice out of 214 typhoid patients, jaundice was never severe and the highest serum bilirubin was 2.4 mgm per 100 ml. Cases are on record where jaundice has been noticed in the first week associated with haematuria and other forms of haemorrhage and some of these cases were fatal, Rawland (1961).

Enlarged tender liver may be noticed, in some cases of typhoid and paratyphoid but occasionally the illness is of long dura-

tion and the liver is so tender, that amaebic hepatitis may be simulated. In the Abbassia Fever Hospital, 29 per cent of enteric patients had enlarged liver.

TABLE 4 — Enteric Fever Presenting as Amaebic Hepatitis

Case No.	Age (yrs.)	Sex	Diagnosis		L.C.
			Bl. culture	Widal	
1	13	M	T	THO 1/500	5800
2	32	F	—	PA 1/500	3600
3	16	M	PA	PA 1/500	
4	14	M	PA	PA 1/160	8900
5	20	M	—	TO 1/640	9750
6	12	M	T	TOH 1/40	6700

Abu Ghareeb (1968), stated that as a part of the intestine, the appendix when affected was usually painless and symptomless, unless it was perforated giving rise to signs and symptoms of peritonitis. He attributed the tenderness which might be elicited in the region of the appendix in severe typhoid cases to be due to the enlarged and inflamed regional mesenteric lymph nodes where local rigidity in the right iliac fossa was definitely lacking. These cases are not uncommon and are almost always identified during operation in general hospitals. Headache and low leucocylic count, direct the attention of the physician to typhoid infection.

Muscle and joint pains may be the main presenting symp-

toms. Muscular and general body aches may be so prominent that cases may simulate influenza especially in winter time. Enteric patients in whom widal test is negative and who complain of sorethroat, acute tonsilitis, muscular pains, arthritis or myocarditis may be misdiagnosed as rheumatic fever. This is further supported if some cases have a functional murmur which may closely simulate that of mitral regurgitation. Moreover, Laboratory investigations such as erythrocyte sedimentation rate (E. S.R.) and anti-streptolysin O (A.S.O.) which are commonly performed in such patients may be misleading. In fact, abnormal readings of these tests, indicate only the presence of an inflammatory condition and are not specific for rheumatic fever. Patients suffering from illnesses which simulate rheumatic fever may, by chance, have an elevated streptococcal antibody titer especially when there is a high incidence of streptococcal infection in the community. In Egypt, Sorour et al. 1968, reported that the incidence of recent streptococcal infections as indicated by the A.S.O. titer of more than 250 Todd units per ml. was 30 per cent among primary school children in Qalyub district (near Cairo). An investigation carried out by me in association with Dr. F. Abdel Wahab, Dr. F. Hetta, and Dr. A. Fadel in Abbassia Fever Hospital has shown that abnormal readings of these tests are quite frequent in enteric patients (under press).

Bradycardia is not commonly observed in enteric patients. Only 29 percent of Abbassia Fever Hospital enteric patients showed bradycardia.

Nervous symptoms and signs may be the presenting features in enteric cases. Headache may be severe enough to simulate meningitis especially when it is accompanied with signs of meningeal irritation. Sometimes typhoid or paratyphoid organisms cause true meningitis. Last year, Dr. Wagih reported the isolation of S. typhi organisms from the C.S.F. of a patient with meningitis admitted to the Abbassia Fever Hospital Huckstep (1962) and Ramsay (1967) found that meningeal signs were more frequent in children. Mental symptoms may dominate the clinical picture and the patient may present as confused, stuperous, psychotic or comatose.

Bacteriological examination may reveal dual infection in some cases. This has been recorded in 5 patients during the last two years in the Abbassia Fever Hospital. A summary is shown in tables 5 and 6. Two years ago, Dr. R. Hablas and Dr. B. Attia had isolated both S. typhi and S. paratyphi A from the urine, in two of the chronic urinary carriers included in my study.

TABLE 5 — Double Infection in Enteric Patients

Case No.	Age	Sex	Bilharzial Inf.	Isolated organisms
1	12 yrs	F	+	S. typhi + S. paratyphi A
2	10 yrs	M	—	
3	7 yrs	M	—	
4	12 yrs	F	—	B. melitensis + S. typhi
5	12 yrs	M	+	

TABLE 6 — Double Infection in Enteric Patients

Case No.	Culture			Widal	W.B.C.	Relapse Blood culture
	Blood	Stool	Urine			
1	PA + T	—	—	—	4580	—
2	T	PA + T		OH1 640	4210	+ PA
3	PA	—	—	O1 640 A1 20	4140	+ T
4	T B. melit.	—	—	O1 640	3800	—
5	T . B. melit.	—	—	O1 160 A1 640	12200	-

In the Abbassia Fever Hospital we found that a persistently negative or low widal titer was noticed in 7 per cent of typhoid, and 60 per cent of paratyphoid patients. Thus it is evident that a negative widal does not exclude enteric infection. Moreover a normal or increased leucocytic count is not against enteric fever. Our results also showed that leucocytic counts between 4000 — 10000 per c.mm. were noticed in 78 per cent and 6 per cent of cases had a leucocytic count above 10000 per c.cm.

Lastly, when chloramphenicol is irregularly administered or given in low doses, the clinical picture may change its usual pattern. The temperature will not subside in due time after administration of the antibiotic; We came across several of such cases and the patient was cured after giving the drug in proper doses. We have to remember that low dosage may lead to the development of drug-resistant strains.

In the Abbassia Fever Hospital our colleague Dr. Wagih recorded the unusual presenting features in 20 typhoid and paratyphoid cases out of 147 enteric deaths during 1968 and 1969, as it is shown in table (7).

TABLE 7 — Initial Diagnosis in 20 Enteric Patients in whom Enteric Infection was proved P.M. Abbassia Fever Hospital 1968-1969.

Gastro-enteritis	3 cases
Rheumatic fever	3 »
Infective Hepatitis	1 »
Tonsilitis	2 »
Bronchitis	2 »
Encephalitis	8 »
Paraplegia	1 »

Wrong diagnosis of enteric fever may lead to disasterous results. Administration of salicytates and corticosteroids when given erroneously to enteric patients raises the incidence of intestinal haemorrhage and/or perforation. From the epidemiological point of view, unrecognized enteric patients will lead to the spread of the disease as well as the increase in the number of undetected carriers.

In conclusion, in countries, where enteric infections are endemic, proper bacteriological and serological investigations must be carried out in every case presenting with fever lasting for more than one week, to exclude the possibility of enteric infection. Blood, urine, and stool cultures are the corner stone for bacteriological diagnosis and these may give positive results at any time of the disease especially when the patient is not taking antibiotics. We have to remember that negative serological results do not exclude enteric infection. In cases presenting as meningitis, blood and C.S.F. cultures for enteric organisms are strongly recommended to exclude the possibility of enteric meningitis. Enteric urinary infections, must be put into consideration in schistosomal patients with urinary obstructive lesions to detect the enteric carrier state.

During operations, surgeons must look very carefully, for perforated typhoid ulcers, in the ileum, appendix or gall bladder when they come across cases of peritonitis without an evident cause.

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SURGICAL COMPLICATIONS OF TYPHOID

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Enteric fever still remains a pressing clinical problem in the developing tropical countries. The milder the attack the more liable is a patient to surgical complications, although it must be realized that it is not only in the ambulant type of case that these complications occur. Surgical abdominal complications manifest themselves about the beginning of the third week of the malady. There are two of these complications which are dramatic in onset namely Haemorrhage and perforation, the former does not need any surgical intervention. Cholecystitis may complicate typhoid either in the acute or chronic form.

The following table presents the most important surgical complications encountered.

INTESTINAL PERFORATION

It constitues one of the most alarming and dreaded catastrophes of typhoid. It leads to peritoneal irritation the signs and symptoms of which are somewhat different from peritonitis due to other causes. This is the cause of misdiagnosis or late diagnosis. However, a successful outcome depends upon early

Surgery in Typhoid

I	Intestinal perforation	0.65%
II	Cholecystitis	5%
	a. acute	
	b. chronic	
III	Arthritis	0.1%
IV	Fibrofascitis	0.1%
V	Thrombophlebitis	6%
VI	Abscesses	6%

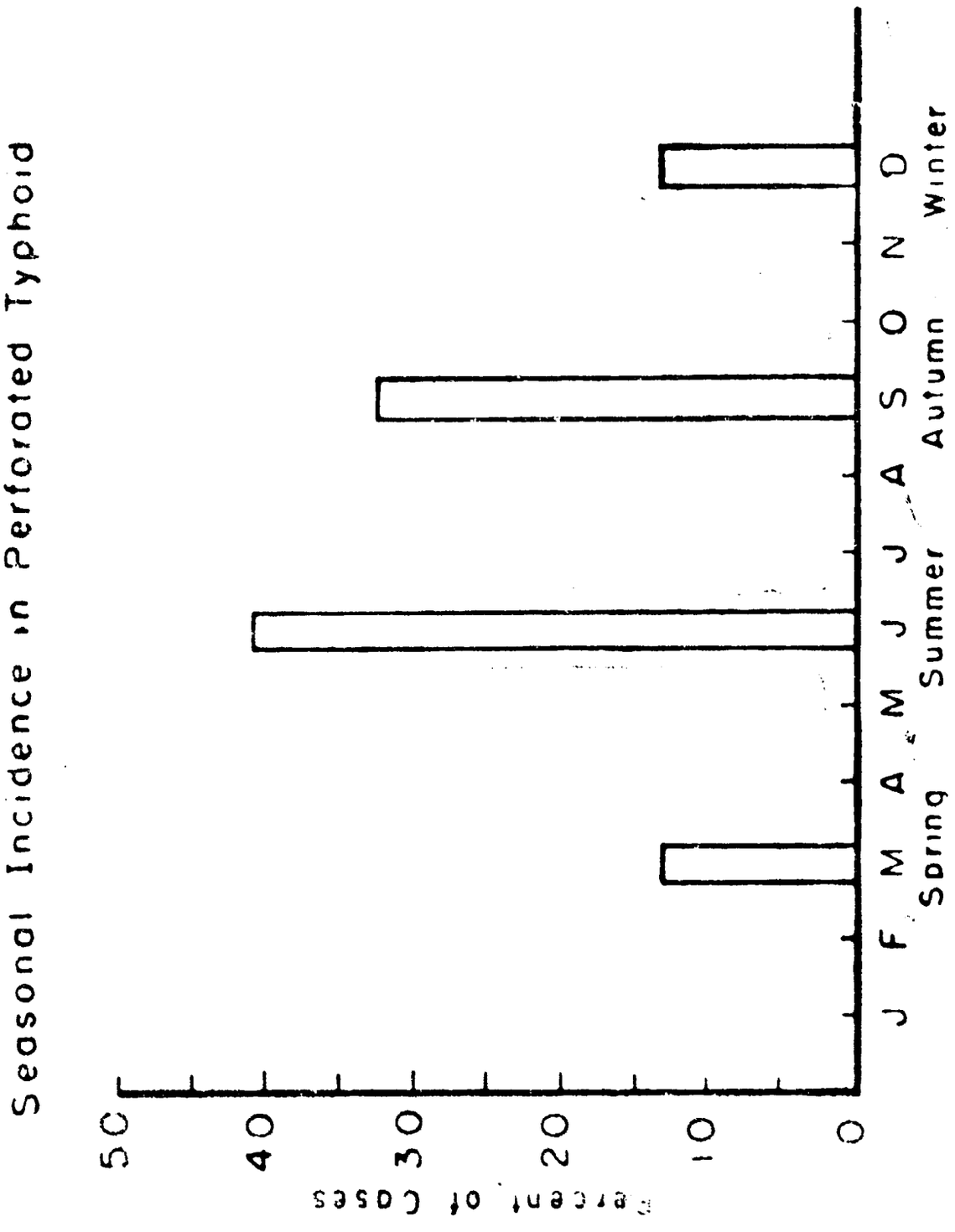
recognition, careful preoperative preparation, timely operative intervention and a guarded judicious post operative management.

My presence for eight years in the field of tropical surgery enabled me to do a retrospective and prospective study of one hundred and sixty eight cases of typhoid perforation encountered in the surgical section of Abbassiah Fever Hospital. That material for study occupied the period between the year 1956 and 1963. All but eight cases were operated upon in the surgical section of the hospital. The latter were subjected to the conservative treatment adopted by Huchstep 1962 due to the lack of personal couscent. All the eight cases died of fulminating general peritonitis as revealed at necropsy.

Total Incidence: In my cases it was 0.65%. Ramly 1952 gave an incidence of 3%. The highest incidence recorded 16.7% was by Archmpong 1969 from Ghana. This high incidence may be explained on Socio economic basis.

Sex Incidence: 70% of the cases belong to the male sex. Preponderance of the male incidence conforms with the reports of Archampong 1969, Huchstep 1962, Li and others.

Age Incidence: The youngest age recorded was four years


Seasonal Incidence in Perforated Typhoid
Percent of Cases
50
40
30
20
10
0
J F M A M J J A S O N D
Spring
Summer
Autumn
Winter

and the oldest was fourty one years. The maximum incidence ranged between fifteen and twenty five years.

Seasonal Incidence: The maximum rise was 40.9% during the summer and 31.8% during the autumn. This was followed by a marked decline during the winter and spring seasons where the incidence was 13.6% in each. The curve shows here two peaks.

Incidence in cases treated previously by chloramphenicol : Fourty five precent of the cases were under full and proper dosage of chloramphenicol before occurance of the perforation. The post operative course of these cases was uneventful and recovery was achieved.

Classification of our cases: According to the time of appearance of the perforation we can describe three clinical groups:-

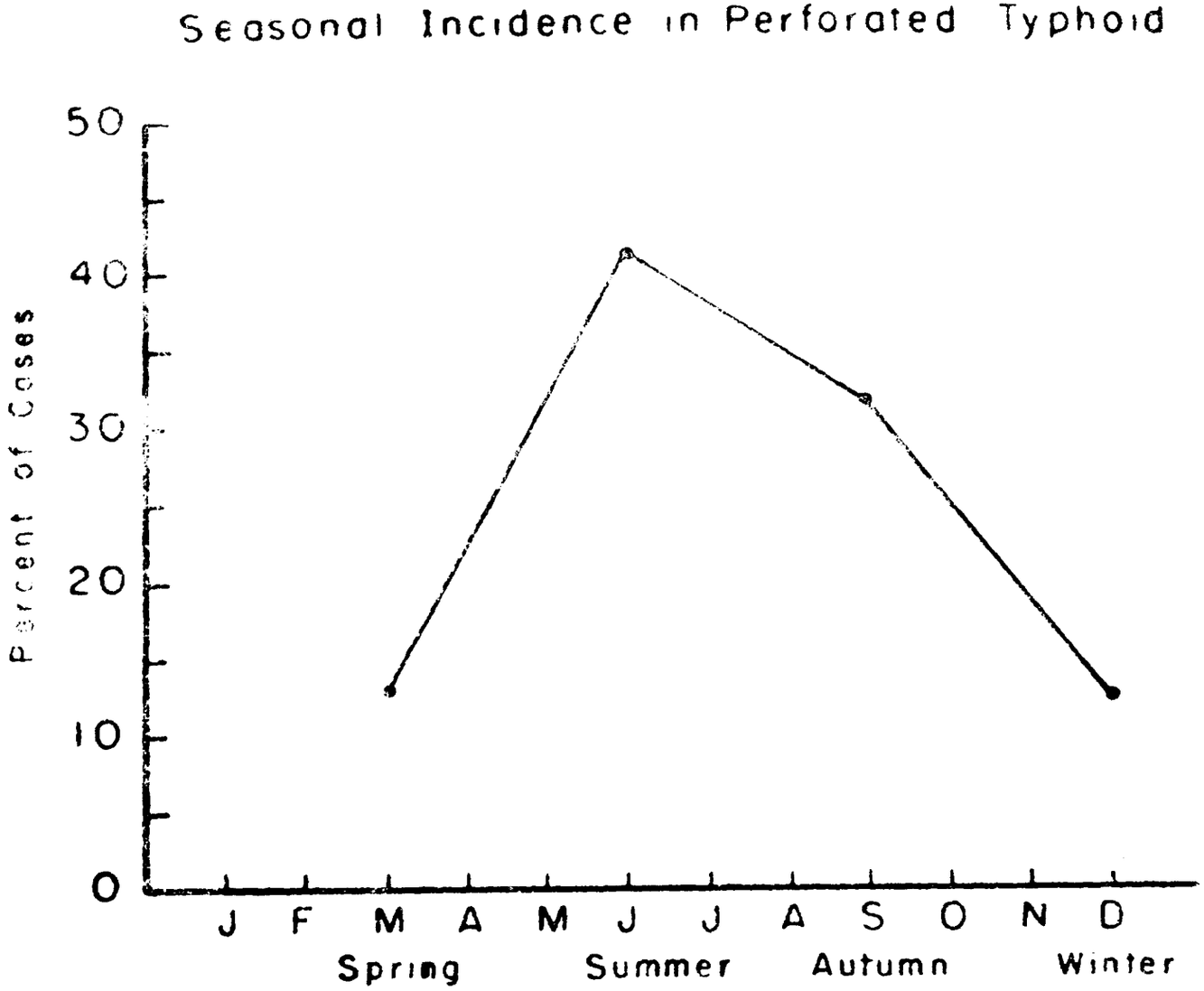
1. In 80% of the cases it occured during the beginning of the third week of the fever.

2. In 4.5% it appeared in a relapse.

3. 16.3% were ambulatory cases where the pain of the perforation was the first complaint of the patient.

ANALYSIS OF THE SYMPTOMATOLOGY

The symptons and signs of peritoneal irritation are modified by the previous anaemic, toxic and hypoproteinemic condition of the patient. In some of our patients we found the combined effects of biliary and septic peritonitis. In the latter cases we encountered intraperitoneal biliary extravasation without macroscopic perforation of the gall bladder. It may be explained by the theory of leakage through microscopic breaches. Moreover in some of the cases opened with a provisional diagnosis of perforation, a serous peritoneal exudate was found in association with areas of impending perforation, the so called leaking ulcers. Other cases proved at laparotomy to be due to


Seasonal Incidence in Perforated Typhoid
Percent of Cases
50
40
30
20
10
0
J F M A M J J A S O N D
Spring
Summer
Autumn
Winter

other lesions in which perforated appendix heads the list. On the other extreme the symptomatology may be so typical but on exploration neither a perforation nor a peritoneal exudate was found. In these latter cases the confusing symptomatology was due to any of the following causes.

1 Typhoid mesenteric — lymphadenitis.

2. Zenker's degeneration of the rectus abdominis muscle associated with rupture of the inferior epigastric vessels.

3. Basal pleurisy.

Here were are stating the symptom complex as gathered from one hundred and sixty eight cases. All but 8 cases were subjected to the operative treatment in the surgical section of the Fever Hospital. The credit was due to my alert colleagues who referred the cases early to me.

1. *Collapse:* This peripheral circulatory upset marked the onset in 85% of the cases. It ushers by pallor, dyspnoea, relative tachycardia and the patient becomes drenched in cold sweat and assumes an apathetic complexion. The pulse and temperature require some consideration.

a. *The pulse:* Becomes rapid and thready. It ranges between 100 and 120 per minute. Although it is not so rapid, it is marked here in contrast with the previous typhoid bradycardia. At the same time irregularities e.g. pulsus alternans may occur and indicate an ominous prognosis.

b. *Temperature:* With the advent of the complication the temperature dropped suddenly usually in association with a rigor, and may remain low up to six hours afted to rise again later at most to 38.5°C. As a matter of fact rigors in typhoid indicate one of the serious complications namely perforation, haemorrhage or thrombophlebitis.

II. *Abdominal Pain:* A sudden stabbing pain in the lower abdomen was the first thing noticed by our patients in 90% of the cases. It is the parietal somatic pain of peritoneal irritation

and is intensified by straining and movement. A constant observation is that the patient resents sleeping on his back and prefers recumbency on his left side. For the first six hours, this is usually the only complain. In 10% of the cases a sudden onset of tenesmus and stranguiy, in otherwords signs of pelvic peritoneal irritation marked the onset of the complication. In the latter cases a misdiagnosis of cystitis or proctalgia due to rectal troubles may be made.

III. *Vomiting:* The early reflex vomiting which is found in other types of peritonitis was not a prominent feature, it was observed only in 10% of the cases and was very slight. To my mind it should not be insisted upon for the diagnosis of the case, otherwise we lose our cases. This is because the later faeculent regurgitant vomiting means irreversibility of the condition.

IV. *Bowel Action:* Strangily enough constipation was not the rule but 85% of the cases continued to pass faeces and flatus up to twelve hours after the onset of the perforation. In 70% of the cases it was preceded by an attack of diarrhoea.

V. *Haemorrhage :* The cataclysmic combination of haemorrhage and perforation occured only in 4% of the cases.

V. *Abdominal Rigidity:* Was not marked except in few of the ambulatory cases. Apart form that it was slight and in severe toxic cases it was absent altogether. This is due to the toxic, moribund condition of the patient, who fails to react in the normal way.

VII. *Abdominal Distension:* It was marked from the inception. It seemed to be an aggravation of the previous typhoid meteorism. There's encroachment on the liver dullness but this is a late sign. In some of the cases subjected to conservative treatment, a skiagram of the abdomen revealed a gas crescent under the cupola of the diaphragm.

VIII. *Shifting Dullness:* It is stated to be condemned for fallacies occur in relation to this sig

INVESTIGATIONS

1. *Scout film:* for the abdomen to detect pneumoperitoneum.

2. *Widal Reaction:* was positive in 80% of the cases, most of which were paratyphoid.

3. *Total and differential leucocytic count:* Leucopenia was present in 5% of the cases.

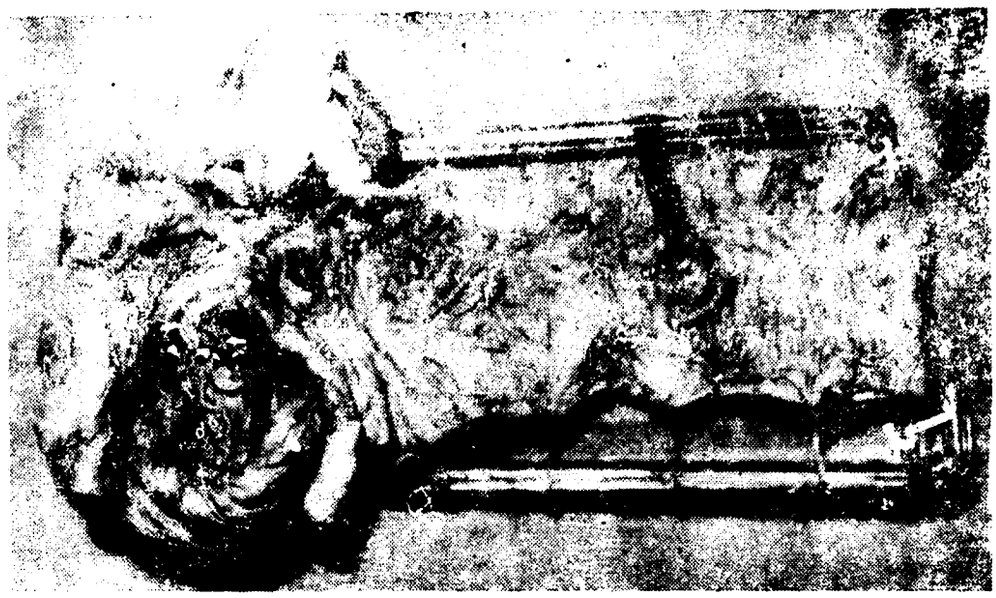
4. *Estimation of the electrolyte pattern*: Disclosed both hypochloraemia and hypokalaemia.

OPERATIVE FINDINGS

I. *Site of the Perforation:* This was the lower reaches of the ileum on the antimesenteric beder where a lot of payer's patches are encountered.

II. *Number of the Perforations:* In 80% of our cases it was solitary. In the rest two or more were encountered. In an interesting cases of undoubted typhoid actiology a perforation was in the ileum and another one at the middle of the vermiform appendix. The rest of the gut showed the typical appearance of typhoid enteritis. That is what we can call typhoid appendititis of a non obstructive nature.

III. *Size of the Perforation* : It varied from 1/4 — 1/2 of an inch in diameter with extensive zone of hyperaemia around. In some of the cases the perforation was so tiny and could only be made apparent by gently squeezing the gut to dislodge the covering fibrin. Other areas of congested peyer's patches which appear as thinned fiery red areas on the surface of the gut and covered with fibrin flakes, when encountered should be treated as areas of threatening perforations and should be infolded and covered with free omental grafts. This is because they are future potential dangers in the immediate postoperative period. The rest of the ileum around is found congested, badly friable and resembles wet blotting paper. Distention of the gut was a marked feature in all the cases. Intestinal clamps



Perforated typhoid ulcers

should be discarded and substituted by the hands which are the most gentle clamps. These precautions have reduced the occurance of postoperative faecal fistula to 4%.

POST OPERATIVE MANAGEMENT

Apart from the routine management and the specific antibiotic cover I gave cortisone in the form of prednisolone intramuscular injections for the first three to four post operative days with dramatic results for the following reasons:—

a. It combats suprarenal depletion as in some of the fatal cases we found haemorrhage of both adrenals at necropsy.

b. It improves the general condition and morale of the patient during that period of stress.

c. It combats toxaemia.

d. Some workers assume that an autoimmune process is responsible for perforation and hence the use of cortisone has got a role.

e. It definitely aids the early restoration of intestinal motility and prevents the development of the bread and butter adhesions. Recent literature did not prove that it might precepitate typhoid perforation of the pathological ulcers. The use of that regimen routinely in our cases reduced the morbidity and mortality rates to 35%. The highest mortality figures were in cases operated upon more than 48 hours after the perforation. In a nut shell operation is the only treatment and conservative treatment is mentioned to be condemned.

THE GALL BLADDER IN TYPHOID

At some time in the course of perhaps of every typhoid fever, organisms are present in the bile. They may during the febrile period cause no symptoms of typhoid or they may occasion a fully developed acute obstructive cholecystitis. A patient with no signs of cholecystitis during the fever may develop gall stones years later, and sometimes the typhoid bacillus may be cultured from these. Even if cholecystitis does not occur, the organisma may after an attack of typhoid fever continue to grow in the gall bladder. This is one of the explanations of the typhoid carrier. All these gall bladder complications of enteric fever are more common in paratyphoid than in typhoid infection.

The treatment of typhoid cholecystitis differ in no respect from the treatment of any other gall bladder infection. It must be remembered however, that when acute obstructive cholecystitis develops in the course of typhoid fever or soon after, it, there are likely to be few limiting adhesions and there is considerable risk of spreading biliary peritonitis if perforation occurs. The cases of the typhoid carriers are sequestrated until they consent to operation. The operation advised is cholecystectomy and drainage of the choledochus until organisms disappear from the biliary discharge. If typhoid or paratyphoid bacilli are not isolated from the removed gall bladder, the subject of operation is likely to remain a typhoid carrier. Even when active typhoid cholecystitis is demonstrated in the removed organ, there is no guarantee that infectivity will cease. There is no case recorded

previously in the literature of typhoid ulceration of the gall bladder in areas of lymphoid aggregations analogous to those in the ileum. I came across such a case during the year 1963 among the typhoid cases of the fever hospital. The patient was a male thirty five years old and was admitted as a case of suspected fever. The case proved by culture and widal to be one of typhoid and received the specific therapy. Two weeks later he experienced a sudden severe abdominal pain with retching, vomiting and collapse. On examination there was a slight conjuctival tinge of jaundice and a board like rigidity and immobility of the upper abdomen. The provisional diagnosis was peritonitis of upper abdominal origin. After resuscitation laparotomy disclosed biliary peritonitis, a gall bladder apparently normal on external examination and three tiny perforations near Hartman's pouch. Cholecystictomy and drainage was instituted, but the patient died twenty four hours later from severe biliary peritonitis. At necropsy the gall bladder disclosed five longitudinal ulcers similar to those found in the ileum and three of these perforated. There were no stones, no obstruction neither in the duct system nor at the splincter of oddi. Hisotlogically the ulcers were found to occupy areas of lymphoid aggreagations. Bile culture was positive to typhoid. The lower ileum showed a typical typhoid ulcer.

DISTANT COMPLICATIONS

ARTHRITIS

It may occur in the later weeks of the attack and produces very litle pain owing to the weak condition of the patient's muscles precluding spasm around the affected point. Such cases rarely proceed to suppuration and are frequently diagnosed when the patient leaves the bed and is found that some joint often the hip is dislocated. The differential diagnosis lies between the many other forms of subacute arthritis. Treatment is as any other type of acute arthritis with the addition of chloramphenicol.

FIBRO FASCITIS

It is a rare complication and is usually not manifested till late in the disease or during convalescence. It is manifested by pain and stiffness in the area affected, usually in the neighbourhood of the spine (typhoid spine) or in the soles of the feet when flat foot may result. It should be stated that some authorities regard typhoid spine as due to an osteo chondritis, but X-rays give no positive evidence that this is the case. Treatment is by rest and physiotherapy.

THROMBOPHLEBITIS

Most of the cases are of the nature of phlebotrhombosis which passes unnoticed by most practitioners. It takes the form of calf muscle tenderness, slight oedema of the leg and foot or a sustained low grade temperature inspite of the proper antibiotic treatment. The minority are cases of thrombophlebitis which are rare. The inferior vena cava or the fenoral veins may be affected. I use to treat these cases with massive anticoagulants and early movement to guard against the development of post phlebitic oedema and swallen leg which is a bugbear to the patient and a problem to the physician.

ABSCESSES

These may affect the bones or cartilages in any part of the body, but probably the most common sites are the costal cartilages and the tibia where the development of a painful bony swelling during or after the attack of typhoid fever should always arouse the suspician that there is an abscess in the cartilage or bone. There is usually no sign of any local infection other than the swelling mentioned, nor is there any general rise of temperature but these patients may suffer from an earthy complexion, marked anaemia and a feeling of lassitude and lack of fitness.

An X ray examination will reveal an irregular cavity with surrounding induration in the bone in chronic cases. If left alone

these abscesses gradually increase and my often discharge on the surface, when the fluid wil contain active typhoid bacilli capable of causing a severe and extensive epidemic of typhoid.

Treatment: The proper treatment is to open the abscess, curette out the cavity and pack it with chloramphenicol solution 5%. All dressings should be burnt.

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WIDAL AND BLOOD PICTURE IN THE DIAGNOSIS OF TYPHOID FEVER

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The definitive diagnostic procedure for enteric fevers is the isolation of salmonella typhi or paratyphi from the blood, but still serologic tests are widely used. Schroeder (1968) stated that serologic tests for enteric fevers are non-specific, poorly standardized, often confusing and difficult to interpret. He considered the titre for O antigen to be the only meaningful value in the diagnosis of enteric fever (H titre being very variable). Even this (O antigen titre) may be suppressed by early treatment with antibiotics or elevated by immunization. Lack of standardized commercial antigens and cross reactions with other salmonellae further confuse interpretation of these tests. Abu-Ghareeb (1968) from Cairo found that Widal reaction is well known to be most misleading, evidenced by the large number of its positivity in other diseases in the same extent as its negativity in typhoid cases and these observations were frequently assured documentally by the post-mortem examination. He stated that one of the causes of increased statistical data of typhoid in the United Arab

Republic is the dogmatic follow of the positive widal reaction at any degree in diagnosis.

It has been found that a certain proportion of individuals in any community have in their sera antibodies to one or more of the organisms of enteric group of fevers. The presence of these antibodies is of importance both in epidemiology and in the laboratory diagnosis of these infections. In England approximately 4% of a group of healthy unvaccinated students had an O antigen titre of 1/50 and fewer than 1% had a titre of 1/100 or more (Christie 1969). Immunization with typhoid vaccine causes a transient elevation in O titre and a persistently elevated titre for H antigen. Christie (1969) believes that in a previously vaccinated patient, an O antigen of less than 1/100 is meaningless and that measuring H antigen titre is also of little value.

Elyan (1958) tested 600 apparently healthy individuals in Cairo and he found TO titre up to 1/160 in 4% of the group, TH titre up to 1/320 in 10%, AH titre up to 1/160 in 9%, BH titre up to 1/320 in 10% and CH titre up to 1/320 in 5% of the series (Table I). He stated that these antibodies might have resulted from latent, remote or subclinical infection with these organisms or others sharing the same antigenic factor. Also may be the result of prophylactic inoculation by typhoid vaccines.

TABLE I: Natural Agglutinins in Egyptians

	Elyan		El.Ramli	
TO	1:160	4%	1:125	
TH	1:320	10%	1:500	
AH	1:160	9%	1:250	13%
BH	1:320	10%	1:250	
CH	1:320	5%	1: 50	

El-Ramli found that 13% of Egyptian sera had natural agglutinins against one or more of enteric organisms.

The aim of this work is to evaluate the efficiency of widal agglutination test and blood picture in the diagnosis of enteric fever in the United Arab Republic as it is widely used here.

MATERIALS AND METHOD

During the period between 1965-1969, 402 acute enteric fever cases were included in the study. All patients were admitted to the Government Fever Hospital, Abbassia, Cairo, U.A.R. Widal agglutination test and complete blood picture were done in NAMRU-3 on admission and at weekly intervals. The somatic antibodies for S. typhi (TO) and flagellar antibodies for S. typhi (TH) and S. paratyphi A (AH) were only evaluated in all patients. Commercially prepared (Lederle) antigens were used to determine antibody titre.

Criteria for inclusion in the study were :

1. No previous history of immunisation by TAB vaccine.

2. No previous administration of chloramphenicol before admission to the hospital.

3. Positive blood culture for S. typhi or S. paratyphi A bacillus.

4. Three consecutive weekly blood samples for widal agglutination test and complete blood picture.

5. No steroid administration during treatment.

Patients were assigned in a rotational fashion to one of the following drug regimes:

i. Chloramphenicol 50 mgm kg body weight daily.

ii. Ampicillin 100 mgm/kg body weight per day. Drug was given until temperature dropped to normal and continued for 7 days apyrexial.

Of a total 402 positive blood culture enteric cases, 311 were due to typhoid bacillus and 91 due to paratyphoid A bacillus (Table II). Two hundred thirty three cases (58%) were males and

TABLE II: Sex and age distribution

	Total	Sex		Age in Years			
		Male	Female	> 5	5 — 15	15 — 40	<40
Typhoid	311	166—53.5%	145—46.5%	27—8.7%	210—67.5%	73—23.5%	1—0.3%
Paratyphoid A	91	67—73.5%	24 26.5%	3—3.3%	55—60.5%	31—34 %	2—2.2%
Total	402	233—58 %	169—42 %	30—7.5%	265—66 %	104—25.8%	3—0.7%

169 patients (42%) females. As regards age distribution, 30 cases (7.5%) were under 5 years, 265 (66%) between 5-15 years, 104 (25.8%) between 15-40 years and 3 (0.7%) were above 40 years.

One hundred fifty nine patients (40%) were admitted to the hospital within the first week of the disease, 203 cases (50%) in the second week and 40 cases (10%) more than 2 weeks duration. One hundred seventy one cases (42.5%) had positive admission stool and or urine culture for S. typhi or S. paratyphi A. One hundred and sixty seven patients (41.5%) had parasitic infestations in the form of bilharzia, ascaris, giardia and hook worm infection. Chloramphenicol was given to 152 cases (38%) and ampicillin to 250 patients (62%).

Out of the total 402 patients, 39 (10%) were considered severe (Shoeb 1969).

RESULTS OF WIDAL AGGLUTINATION TEST

1. Non complicated cases:

Knowing the upper limits of natural agglutinins in Egyptians (Elyan 1958), we considered a titre of 1/320 or above to be significant for TO and AH antibody and a titre of 1/640 or above significant for TH antibody. The admission antibody titre in 276 moderate typhoid cases is shown in Table III a and b. Significant TO antibody titre was found in67% of the total cases while negative TO titre was observed in 13% of patients. Fifty six percent of patients admitted early in the first week had a significant widal titre, 71% in the second and 87% in the third week of illness. A significant TH titre was observed in only 7% of 276 moderate typhoid patients, taking into consideration the results of widal test during 3 weeks of disease.

In the 35 severe typhoid cases (Table IV a and b), a significant TO antibody titre was found in 85% of cases while a significant TH antibody titre was found in 28% of the patients (including the results of widal test in 3 weeks).

Out of a total of 311 typhoid patients, AH antibody titre was

TABLE III a: Admission To Titre in 276 Moderate Typhoid

Week of Admission	No. Cases	Negative titre		Non-significant titre		Significant titre					
				1:160		1:320		1:640		More than 1:640	
First week	110	19	17.3%	29	26.4%	15	13.7%	39	35.4%	8	7.2%
Second week	143	16	11.2%	25	17.5%	25	17.5%	71	49.6%	6	4.2%
Third week	23	1	4.4%	2	8.6%	—	0%	20	87 %	—	0%
Total	276	36	13.0%	56	20.0%	40	14.5%	130	47 %	14	5.5%

TABLE III b: Admission TH Titre in 276 Moderate Typhoid

Week of Admission	No. Cases	Negative titre		Non-significant titre				Significant titre			
				1:160		1:320		1:640		More than 1:640	
First week	110	73	66.3%	21	19.1%	6	5.5%	9	8.2%	1	0.9%
Second week	143	98	68.5%	30	21 %	7	4.9%	8	5.6%	—	0 %
Third week	23	15	65.3%	5	21.7%	2	8.6%	1	4.4%	—	0 %
Total	276	186	67.5%	56	20 %	15	5.5%	18	6.5%	1	0.5%

TABLE IV a: Admission TO Titre in 35 Severe Typhoid

Week of Admission	No. Cases	Negative titre		Non-significant titre		Significant titre					
				1:160		1:320		1:640		More than 1:640	
First week	16	1	6.3%	1	6.3%	1	6.3%	10	62.4%	3	18.7%
Second week	16	2	12.4%	1	6.3%	1	6.3%	9	56.2%	3	18.8%
Third week	3	—	0 %	—	0 %	1	33.3%	1	33.3%	1	33.3%
Total	35	3	8.6%	2	5.7%	3	8.7%	20	57 %	7	20; %

TABLE IV b: Admission TH Titre in 35 Severe Typhoid

Week of Admission	No. Cases	Negative titre		Non-significant titre				Significant titre			
				1:160		1:320		1:640		More than 1:640	
First week	16	8	50 %	1	6.3%	2	12.4%	4	25%	1	6.3%
Second week	16	6	37.5%	5	31.2%	1	6.3%	3	18.7%	1	6.3%
Third week	3	—	0 %	1	33.3%	1	33.3%	1	33.3%	—	0 %
Total	35	14	40 %	7	20; %	4	11.4%	8	22.9%	2	5.7%

negative in 239 cases (77%) and gave positive results in a titre of 1/160 in 66 (21%) and 1 320 in only 6 patients (2%). Almost all these AH titres are non-significant.

The admission AH antibody titre in 91 paratyphoid A patients is demonstrated in table V. A significant AH titre was found in 11.5% of the cases. Only 6.5% of patients admitted in the first week had a significant widal titre, 14.3% in the second and 14.2% in the third week of the disease. In the 91 paratyphoid A patients, TO antibody titre was negative in 33 cases (36%) and gave positive results in the titre of 1/160 in 30 patients (33%), 1/320 in 10 (11%), 1/640 in 17 (19%) and more than 1/640 in only one patient (1%). As regards TH antibody titre it was negative in 84 cases (92%) and gave positive results in the titre of 1/160 in 7 patients (8%). All these TH titres are non significant.

Results of taking 3 consecutive weekly agglutination titres are shown in table VI. Out of total 311 typhoid patients, negative TO titres was found in 39 cases (13%) and titres up to 1/160 was found in 58 cases (19%). Four fold increase of TO titres was found in 60% of negative titres and 82% of titres up to 1/160.

Out of total 91 paratyphoid A patients Table VII. negative AH titres was found in 71 cases (78%) and titres up to 1/160 was found in 10 cases (11%). Four fold increase of AH titres was found in 25% of negative titres and 20% of titres up to 1/160.

II. Complicated clinical courses:

In our series we have 16 typhoid relapses and 13 paratyphoid A ones. In 16 typhoid relapses, TO antibody titre was negative in 2 cases (12%), 1/160 in one (6%), 1/320 in 3 (18%) and 1/640 in 10 (64%) patients. In the 13 paratyphoid A relapses, the AH antibody titre was negative in 4 cases (31%), 1/160 in 5 (38%), 1/320 in one (8%) and 1/640 in 3 cases (23%). Comparing the results of widal obtained before and during the relapse we found that in 16 typhoid cases, TO titre was the same in 10 cases (63%), decreased in 4 (25%) and increased in 2 (12%). In the 13 paratyphoid cases, AH titre was the same in 2 cases (15%), decreased in 2 (15%) and increased in 9 (70%) patients.

TABLE V -- Admission AH Titre in 91 Paraphoid A

Week of admission		No. of cases	Negative titre		Non-significant titre		Significant titre					
					1:160		1:320		1:640		More than 1:640	
First week	M	31	25	80.2%	1	3.3%	2	6.5%	—	0 %	—	0%
	S	2	2	100. %	—	0 %	—	0 %	—	0 %	—	0%
Second week	M	42	29	69 %	7	16.7%	4	9.5%	2	4.8%	—	0%
	S	2	2	100. %	—	0 %	—	0 %	—	0 %	—	0%
Third week	M	14	10	71.5%	2	14.3%	1	7.1%	1	7.1%	—	0%
	S	—	—		—		—		—		—	
Total	M	87	67	77 %	10	11.5%	7	8 %	3	3.5%	—	0%
	S	4	4	100 %	—	0 %	—	0 %	—	0 %	—	0%

M = Moderate S = Severe.

TABLE VI — Results of 3 Consecutive Typhoid Widal Agglutination Titres

Widal on admission		Negative				Up to 1:160				Up to 1:320				Up to 1:640				Higher than 1:640			
		No.	Same	Inc.	Dec.	No.	Same	Inc.	Dec.	No.	Same	Inc.	Dec.	No.	Same	Inc.	Dec.	No.	Same	Inc.	Dce.
1st week	TO	20	8	12	—	30	1	27	2	16	2	13	1	49	39	6	4	11	2	—	
	TH	81	50	31	—	22	4	10	8	8	2	3	3	13	7	2	4	2	—	—	2
2nd week	TO	18	7	11	—	26	4	20	2	26	4	18	4	80	63	11	6	9	1	—	8
	TH	104	79	25	—	35	9	10	16	8	—	4	4	11	6	1	4	1	1	—	—
3rd week	TO	1	1	—	—	2	1	1	—	1	—	1	—	21	17	—	4	1	—	—	1
	TH	15	11	4	—	6	2	2	2	3	1	—	2	2	1	—	1	—	—	—	—
Total	TO	39	16	23	—	58	6	48	4	43	7	31	5	150	119	17	14	21	3	—	18
	TH	200	140	60	—	63	15	22	26	19	2	8	9	26	14	3	9	3	1	—	2

Inc. = Increased. Dec. = Decreased.

TABLE VII — Results of 3 Consecutive Paratyphoid A Widal Agglutination Titres

Widal on admission		Negative				Up to 1:160				Up to 1:320				Up to 1::640				Higher than 1:640			
		No.	Same	Incr.	Decr.	No.	Same	Incr.	Decr.	No.	Same	Incr.	Decr.	No.	Same	Incr.	Decr.	No.	Same	Incr.	Decr.
1st week	AH	30	25	5	—	1	—	—	1	2	—	—	2	—	—	—	—	—	—	—	—
2nd week	AH	31	21	10	—	7	2	2	3	4	2	—	2	2	—	—	2	—	—	—	—
3rd week	AH	10	7	3	—	2	—	—	2	1	—	—	1	1	1	—	—	—	—	—	—
Total	AH	71	53	18	—	10	2	2	6	7	2	—	5	3	1	—	2	—	—	—	—

In the three cases complicated by intestinal haemorrhage, the TO titre in the 2 typhoid cases was 1/80 and 1/640 respectively while the AH titre in the third paratyphoid A patient was negative. In the 2 patients complicated by intestinal perforation, the TO titre was 1/640 in one typhoid case and AH titre was negative in the paratyphoid A patient. In the three typhoid patients who suffered from severe haemolytic crisis in our series, the TO titre was 1/1280 in 2 cases and 1/320 in the third. In typhoid bleeding, perforation and blood haemolysis, the antibody titre remained the same as before the incidence of complication We could not demonstrate significant relation between the occurrence of complications and inhibition of antibody titre.

III. Effect ofchloramphenicol and ampicillin on antibody titre:

Figures I and II demonstrate the effect of chloramphenicol and ampicillin on the antibody titre in 311 typhoid patients. It appears that there is no significant difference between the 2 antibodies as regards the effect on the TO and TH antibody titre when considered as a whole. However early in the first week of typhoid disease, chloramphenicol seems to inhibit TO antibody titre as compared with ampicillin. Figure III shows that chloramphenicol probably inhibits AH antibody titre in the 91 paratyphoid A patients.

BLOOD PICTURE

Table VIII shows admission total white blood counts.

(1) Typhoid patients: Leucopenia (below 4000) was observed in 15% of our moderate typhoid cases. The white blood count was between 4-5000 in 22.5%, between 5-7000 in 36%, between 7-10,000 in 20.5% and over 10,000 in 6% of the series.

(2) Paratyphoid A patients: Leucopenia was observed in 19.5% of our moderate paratyphoid A cases. The white blood count was between 4-5000 in 27.6% of cases, between 5-7000 in 32.4%, between 7-10,000 in 12.7% and over 10,000 in 7.8% of the series.

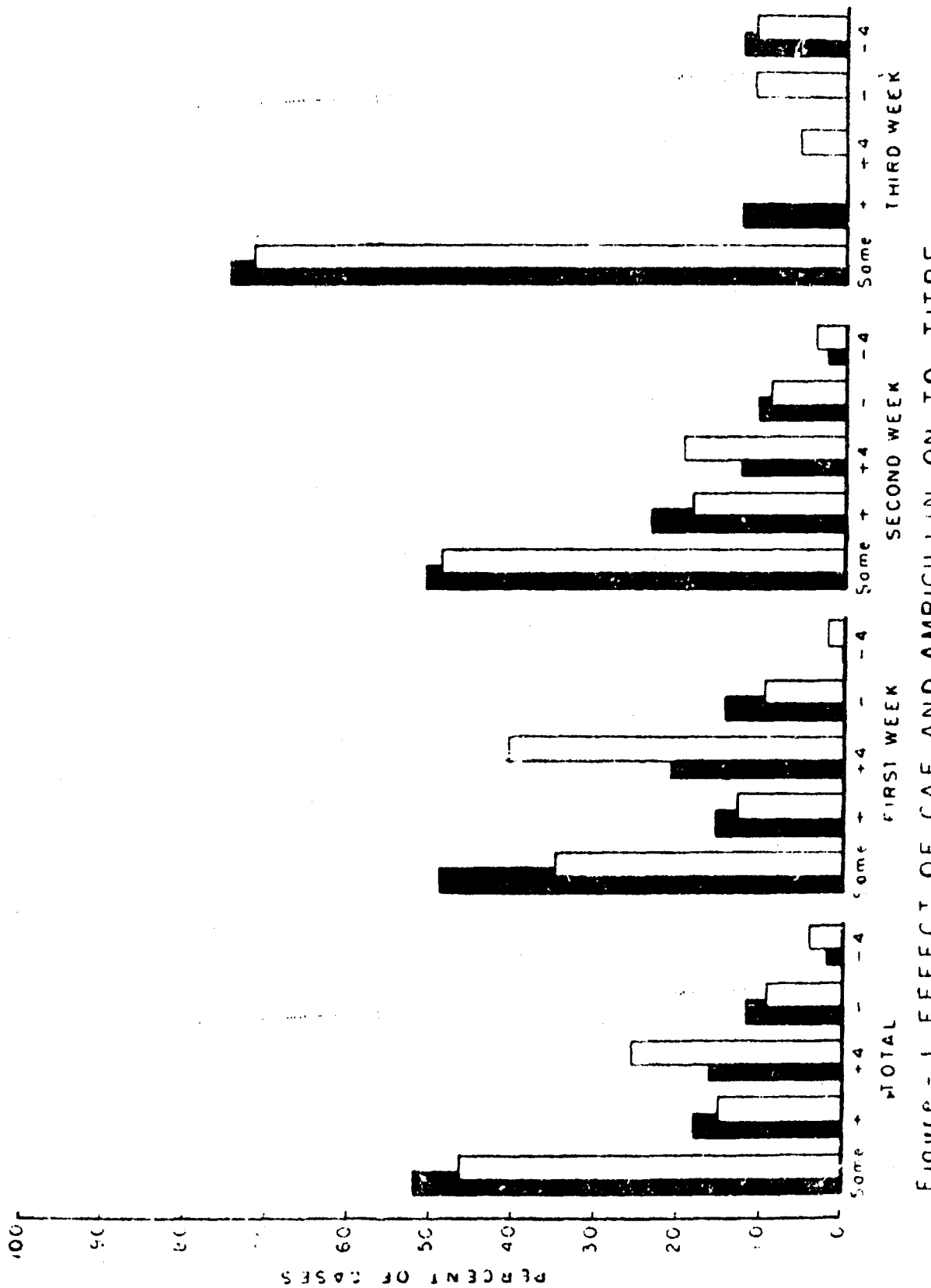

Figure - 1 EFFECT OF CAF AND AMPICILLIN ON TO TITRE

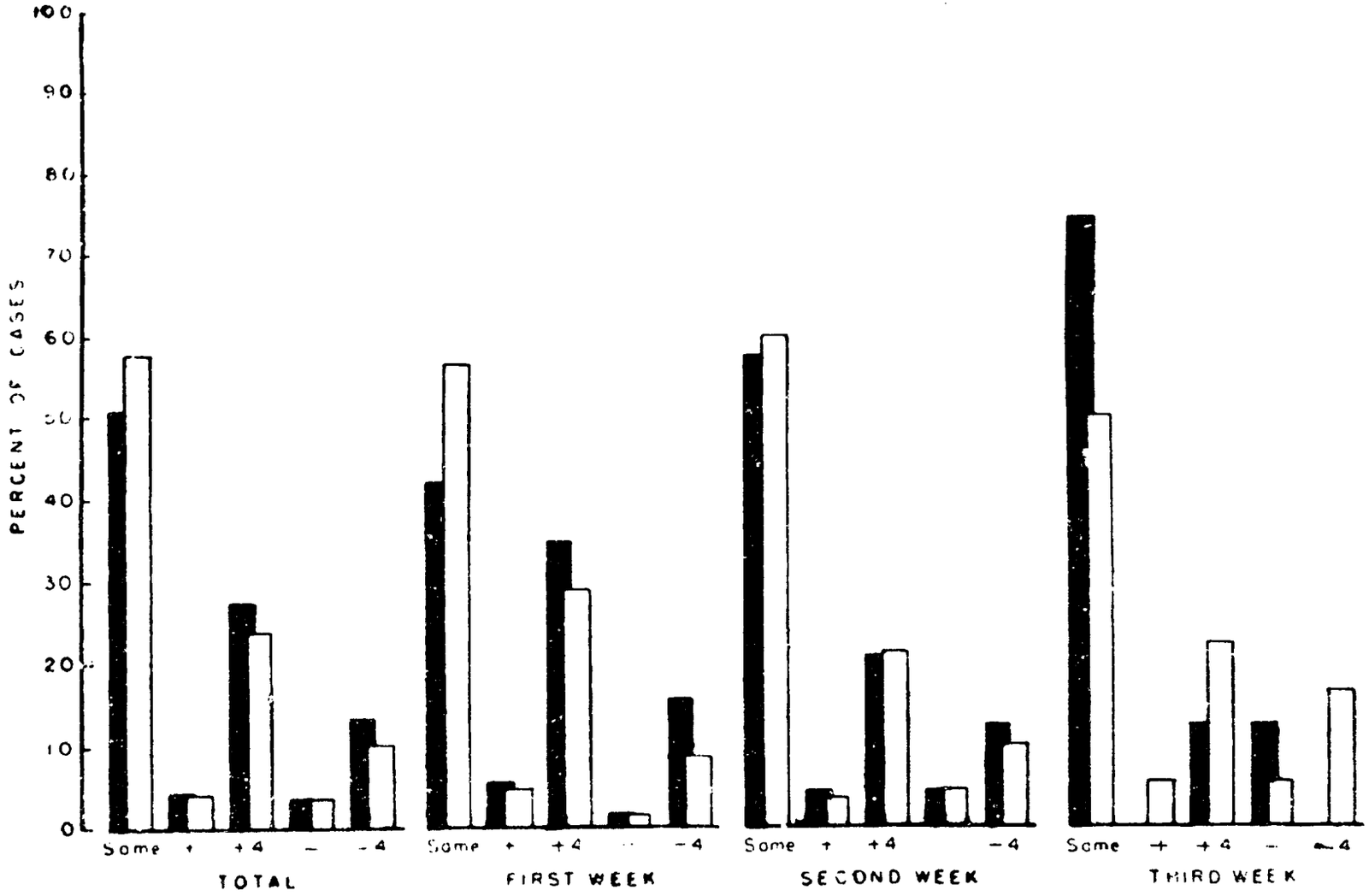

Figure-2 EFFECT OF CAF AND AMPICILLIN ON TH TITRE

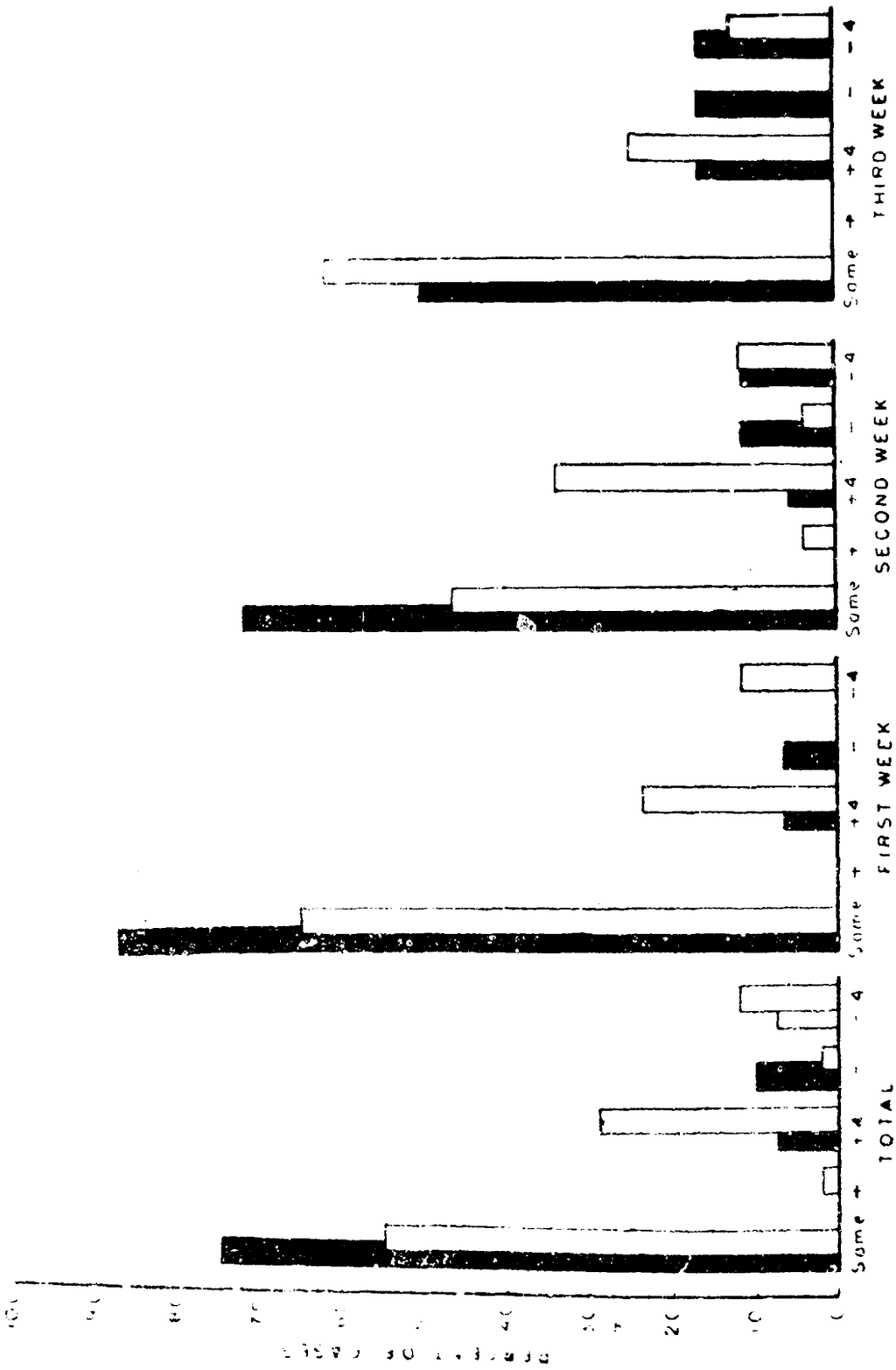

Figure – 3 EFFECT OF CAF, AND AMPICILLIN ON AH TITRE

TABLE VIII

Admission total white **blood** count.

		No.	4000	4—5000	5—7000	7—10,000	10,000
Typhoid	M	276	41—15 %	62—22,5%	99—36%	57—20,5%	17—6 %
	S	35	8—23 %	8—23 %	10—28%	8—23 %	1—3 %
Paratyphoid	M	87	17—19,5%	24—27,6%	28—32,4%	11—12,7%	7—7,8%
	S	4	— 0%	1—25 %	2—50%	— 0%	1—25 %

M — Moderate S — Severe

Relative lymphocytosis (more than 40%) was detected in about half of our moderate typhoid and paratyphoid A cases (Table IX).

On admission no eosinophils could be detected in 84% of our moderate typhoid and 76% of paratyphoid A patients. The rest of the patients with normal or relatively high eosinophilic counts were mostly harbouring intestinal parasites. The majority of the enteric cases showed return to previous level on recovery.

No significant relation was noticed between the incidence of complications and the total white blood count or lymphocytic ratio. There was a definite decrease of eosinophilic count in the peripheral blood independent of the administration of chloramphenicol in most of our complicated cases.

No significant difference was observed between the effect of chloramphenicol and ampicillin on either the total white blood count or the differential lymphocytic count (Table X). A rise of total white blood count was found in about half of our cases while a rise of lymphocytic count occurred in 80% of the patients.

No significant difference could be detected between the effect of chloramphenicol and ampicillin on the haemoglobin and haematocrit value. A drop of haemoglobin and haematocrit of varying degree was found in about half of our moderate cases. (Figure IV). A marked drop of haemoglobin and haematocrit values was shown in enteric cases complicated by severe hemolytic anemia under chloramphenicol therapy.

DISCUSSION

Huckstep (1962) stated that formation of antibodies in typhoid fever is not only circulating (and so giving widal reaction), but also there is cell fixed immunity. Many factors as dosage and virulence of the bacillus, presence of non-motile strains, duration of the disease, previous TAB vaccination, state of immunity in the community and lastly administration of chloramphenicol play a role in the interpretation of the antibody response. Hablas (1970) believes that widal test is a mere evidence of infection and not immunity.

TABLE IX . Admission differential white blood count

		No.	Lymphocytes			Eosinophils		
			<40%	40 -60%	>60%	None	2 -4%	>4%
Typhoid	M	276	135 -49%	118 -43%	23--8%	231—83.6%	44—16%	1—0.4%
	S	35	22 -63%	12 -34%	1--3%	32--92 %	3— 8%	——0 %
Paratyphoid	M	87	45—52%	34 -39%	8- 9%	66--76 %	20 -23%	1—1 %
	S	4	1 -25%	3 -75%	—.—0%	3—75 %	1--25%	——0 %

TABLE X — Effect of Chloramphenicol and Ampicillin Therapy on White Blood Count..

Drug Used	No. Cases	Total W.B.C. after therapy			Lymphocyte % after therapy		
		Same	Increase	Decrease	Same	Increase	Decrease
Chloramphenicol	152	16—10%	91- 60%	45--30%	18—12%	121 -80%	13— 8%
Ampicillin	250	42—16%	129 -52%	79 --32%	20 -8%	201 -80%	29 -12%
Total	402	58—14%	220 -55%	124 -31%	38 -9%	322 -81%	42 --10%

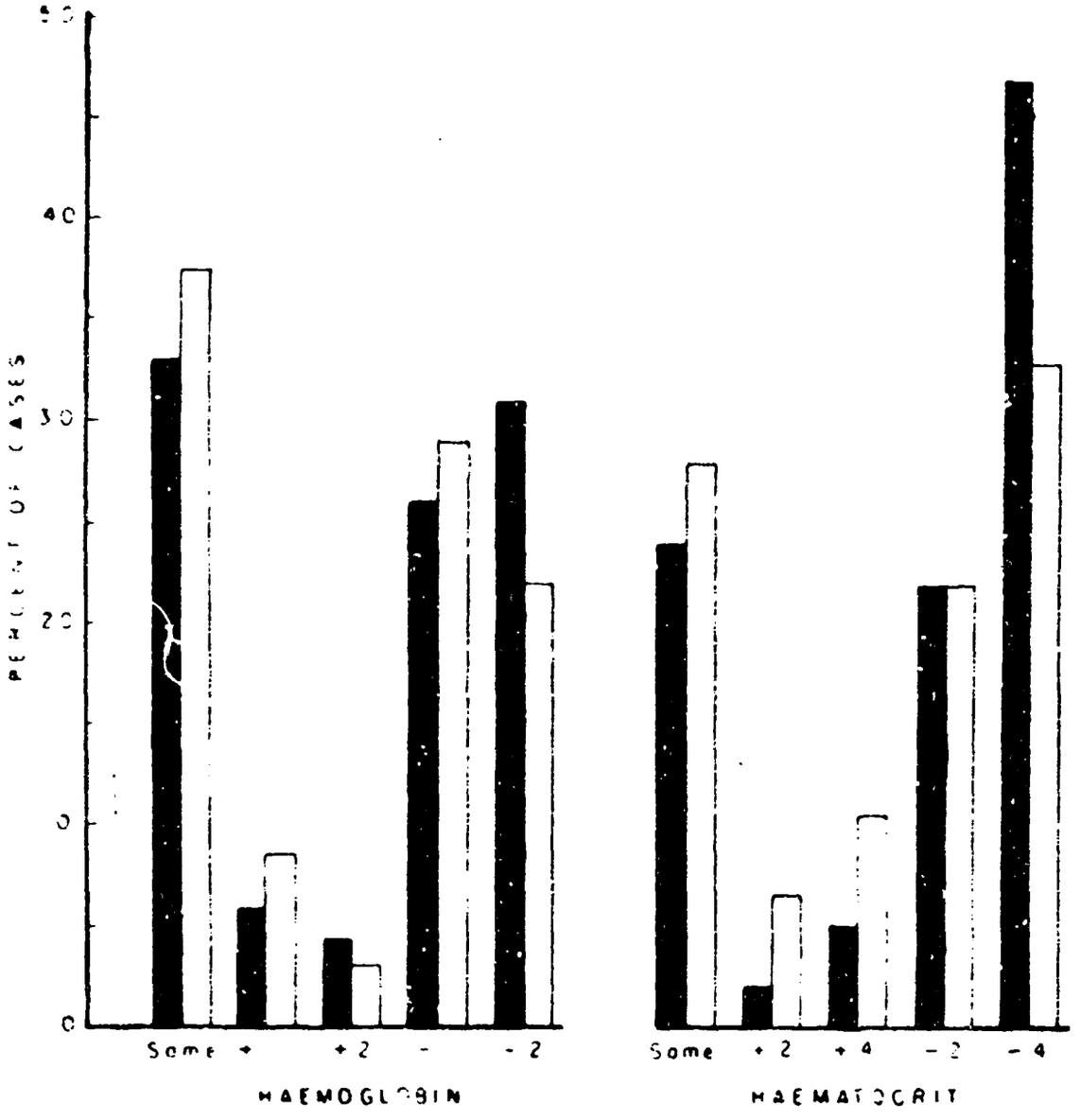

Figure - 4 EFFECT OF CAF AND AMPICILLIN ON HAEMOGLOBIN AND HAEMATOCRIT

We considered a significant widal titre as that above the upper limit of the normal (i.e. for TO and AH a titre of 1/320 or above while for TH a titre of 1/640 or above), as has been stated previously.

A significant TO titre was found in 68% of our typhoid cases, this favours Sood and Taneja (1961) and Robertson (1968) who reported 62% and 55% values respectively. Nevertheless, Nelson (1951) reported 90% significant widal titre in typhoid. TH titre is variable and this agrees with that of Schroeder (1968) Only 5% of our typhoid cases had a persistently negative TO titre all over the disease while 2% of the patients had a repeatedly low TO titre up to 1/160.

A significant AH titre was found in 11% of our paratyphoid A patients. 58% of our paratyphoid A patients had persistently negative AH titre all through the disease, while 2% of the patients had a persistent low titre up to 1/160, most of these cases were schistosomal. The presence of significant TO titres in 31% of our paratyphoid A patients is due to the fact that S. typhi and S. paratyphi A and S. paratyphi B all contain the O factor 12, this explains the cross-agglutination with O antibody in widal test.

So negative or low widal titre is not against the diagnosis of enteric fever when the clinical picture is suggestive of the disease. Stuart (1946), El-Ramli (1950), Kamal (1958), Wilson (1964) and Abu-Ghareeb (1968) found that in some enteric fever cases, no titre elevations are ever documented.

We found that complicated cases were not related to inhibition of antibody formation as measured by widal test. This agrees with the findings of El-Ramli and Robertson (1969). Woodward (1950) stated that relapses are not correlated with the height of O antibody titres.

This work suggested that no significant difference was detected between the effect of chloramphenicol and ampicillin on either TO or TH antibody titre of the total typhoid cases. This finding agrees with that of Robertson (1969) who could not demonstrate that chloramphenicol consistently and significantly

inhibits antibody formation (as measured by widal reaction) in man with enteric fever as has been described in experimental infection by Ambrose (1963) and Weisberg (1964). El-Ramli (1950) and Good and Mackenzie (1950) found that chloramphenicol has little if any interference with antibody formation. On the other hand Jezyna (1966) and Erazo (1964) reported inhibition of antibody response due to chloramphenicol.

Early in the disease, it appears that chloramphenicol as compared with ampicillin inhibits the TO antibody titre. This agrees with the work of Leo Ping (1953), Vorlaender (1955), El-Rooby (1956) and Schroeder (1968) who concluded that chloramphenicol interferes with antibody formation when given early in the disease. El-Rooby (1956) found that administration of chloramphenicol to healthy persons during and after immunization with TAB vaccine had no effect on the agglutinin response. He suggested that the mechanism by which this drug depresses antibody titre in enteric fever is probably through its bacteriotatic action and consequently depression of antigenic stimulation.

No significant difference was observed between typhoid and paratyphoid A patients as regards white blood count.

Huckstep (1962) found that 46% of his patients had a white blood count below 5000 and 9% had a count above, 10,000 favouring our results. Abu-Ghareeb (1968) found that in many typhoid cases, seen in post-mortem especially fulminating and toxic cases, the leucocytic counts were ranging from 10-12,000 with no precise changes in the differential count.

We can say confidently that a normal or increased leucocytic count is not against the diagnosis of enteric fever when the clinical picture is suggestive of the disease.

Relative lymphocytosis was present in about half of our moderate enteric cases. This favours the work of Shoeb (1969). Abu-Ghareeb (1968) found in all typhoid cases that there were definite and precise increase in the younger lymphocytes (up to 20%). He stated that this differential of the differential count, so to speak, may be used as a very rapid way of diagnosis in severe and toxic cases.

Absence of or low eosinophils in the peripheral blood in cases of enterica may be of diagnostic and prognostic value favouring Shoeb's (1969) results.

Serial haemoglobin and haematocrit values' determination is essential in detecting early the serious complication of severe haemolytic anaemia under chloramphenicol therapy. About 5% of our population (Hashem 1970) are glucose-6-phosphate-dehydrogenase deficient. Significant drop of haemoglobin and haematocrit value usually occur before the overt clinical picture of severe blood haemolysis supervene.

SUMMARY

Widal agglutination titre and complete blood picture were done in 402 positive blood culture typhoid and paratyphoid A patients. A significant admission TO titre of 1/320 or above was found in 68% of our typhoid patients while a persistant negative or low widal titre was found in only 7% of cases. A significant admission AH titre was found in 11% of the paratyphoid A cases while a persistant negative or low widal titre was observed in 60% of cases. We consider widal test a suggestive rather than a diagnostic one. The corner stone of diagnosis of enteric fever is the isolation of causative organisms. In our series, 40%, 50% and 10% of the patients respectively had positive blood culture on the first, second and third week of the disease in order. Also 42.5% of our cases had positive admission stools or and urine culture for the causative organism. Rising widal agglutination titre (38% of TO typhoid titre and 22% of AH paratyphoid titre) is diagnostic of the disease.

A normal or increased leucocytic count is not against the diagnosis of enteric fever. Absent eosinophils in peripheral blood was found in 84% of typhoid and 76% of paratyphoid A patients and it is of both diagnostic and prognostic value. Complicated clinical courses were not related to inhibition of antibody titre or the readings of blood picture.

RECOMMENDATIONS

A recent survey for detection of natural agglutinins in the population both in the urban and rural areas is recommended. Also we should use local salmonella strains as an antigen in the widal agglutination test.

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DIAGNOSIS OF TYPHOID FEVER BY HAEMAGGLUTINATION

By

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The determination of specific antibodies by the agglutination of erythrocytes sensitized with extracts of different antigens, was first reported by Keogh, North and Warburton (1947, 1948). Since then, this phenomenon has been applied as a diagnostic test for the evaluation of various bacterial and viral antibodies and also in studies of certain auto-immune diseases.

Spuan (1951, 1952) used haemagglutination for determining Salmonella typhi O and Vi antibodies and demonstrated that the Vi haemagglutination test is about three times more sensitive than the agglutination test performed with living cultures. Neter et al. (1956), working with Salmonella and Shigella, found that haemagglutination gives much higher

titres than direct bacterial agglutination, and Graber and Dodd (1956), applying the test fo enteropathogenic E. coli strains, obtained identical sensitivity by both tests.

More recently the latex agglutination test was introduced and applied for the demonstration of enterobacterial antibodies where specific agglutination is produced by O antibodies present in sera of patients recovering from enteric infections. In a comparative study by Whang and Neter (1963), haemagglutination proved to be more sensitive than the latex test.

The present study was undertaken with a dual purpose : to prove the specificity of the haemagglutination test in the diagnosis of typhoid fever and to apply the test as a routine diagnostic procedure.

MATERIAL AND METHODS

1. *Sera :*

a) Serum specimens from patients : 3 samples from each of 154 patients of different age groups admitted to the Abbasia Fever Hospital over a period of 1.5 years, were taken at weekly intervals. From the 115 typhoid cases, 99 first, 102 second and 99 third samples were obtained and tested. Similarly, from the 39 paratyphoid. A cases, 34 first, 35 second and 32 third samples were collected and tested. Diagnosis of enteric fever was confirmed by blood culture and Widal reaction.

b) Serum specimens from controls, 65 control sera were obtained from the blood transfusion centre of the Ain Shams University Hospitals and also from routine blood samples sent from the Hospital for the Wassermann reaction.

c) Positive control sera obtained by hyperimmunising rabbits. Normal rabbits weighing approximately 2 kgs were inoculated intravenously at intervals of four days by five consecutive doses of 0.25, 0.5, 1.0, 1.5 and 20 ml of the following antigens; a suspension of a living Salmonella typhi

strain VI/1/61, an alcohol-acetone treated suspension of the same Vi strain and a heated alcohol-acetone treated suspension of S.typhi strain 0-901.

d) Standard Burroughs Wellcome antisera against S.typhi (Vi, O and H) and S.paratyphi A, B and C (O and H).

2. *Antigens :*

d) O antigen freshly poured, thick nurment agar plates, 13 cms in diameter, were inoculated with strain 0-901, incubated at 37°C for 24 hrs. The culture was suspended in 0.85% saline heated at 100°C for two hrs. and centrifuged. The sediment was then suspended in absolute alcohol and kept for four hrs. at 37°C. This suspension was recentrifuged, the supernatant discarded and the deposit washed twice with acetone. The deposit was left to dry overnight at 37°C and the powder obtained emulsified with saline and used as antigen in a turbidimetric density equivalent to 1000 million organisms per ml. O antigens were also prepared from S.paratyphi A, B and C strains using the same procedure.

b) Vi antigen 24 hr. agar cultures were harvested in a minimal amount of saline, to which three times its volume absolute alcohol were added. The Vi antigen was then processed in a similar way to the O antigen, except that heating was not performed.

Antigens in the proper dilution were kept at 4°C.

3. *Red cells :*

Human group O cells were used. To every 10 ml. blood, 2ml acid citrate dextrose solution (2 gm disodium citrate, 3 gm dextrose in 120 ml double distilled water) were added. The red cells were centrifuged and washed three times with saline in graduated centrifuge tubes. Final centrifugation was done for 10 minutes at 1000 R.P.M. to pack the cells. Citrated blood could be stored for several weeks at 4°C.

4. *Sensitization of red cells (coating) :*

To one volume of packed cells, 9 volumes of the antigen suspension to be coated on the red cells, were added with shaking. The mixture was incubarted for one hr at 37°C and then centrifuged for 5 minutes at 1000 R.P.M. and the supernatant discarded. Red cells were washed three times with saline and then a 1 % suspension of the coated cells in saline was prepared which would be ready to be used for the haemagglutination reaction. The sensitized red cells can be used within three days of preparation.

5. *Haemagglutination technique:*

The technique was performed in Perspex plates (80 hole, W.H.O.) by the pattern method. All reagents were used in 0.25 ml volumes using an automatic syringe. Sera were inactivated before use by heating in a water bath at 56°C for half an hr. 0.25 amounts of two fold dilutions of the sera were made in saline starting by 1 : 2 up to 1 : 1024. To each dilution was added 0.25 ml of the 1 % sensitized red cells. The plates were placed in an incubator at 37°C and left to settle for one hr., after which the results were read. End points were taken as the highest dilution of serum which produced 50 % aggiutination of the red cells, and the titres were expressed by the reciprocal of this dilution. Hyperimmune rabbit sera and sera from normal controls as positive and negative controls respectively and also coated cells without sera were included in each experiment.

6. *Demonstration of the specificity of the haemagglutination technique :*

S. typhi and S. paratyphi A, B and C rabbit antisera were tested by the haemagglutination reaction against red cells sensitized with S.typhi Vi and O antigens and similarly red cells coated with S.paratyphi A, B and C « O » antigens.

In another experiment to prove the specificity, red cells were sensitized with heat killed suspensions on a 24 hr agar culture of the previously mentioned organisms except the Vi strain.

7. Titration of antibodies in sera of patients and controls :

All sera from typhoid and paratyphoid A cases as well as the controls, were tested by the haemagglutination reaction against red cells coated with S.typhi O antigen. Sera from 53 typhoid and 12 paratyphoid A cases and all controls were tested for agglutination with Vi-coated red cells.

RESULTS

In preliminary studies, using the described haemagglutination technique with hyperimmune rabbit sera, it was found that red cells coated with S.typhi O antigen were agglutinated at a titre of 65536, while Vi antigen coated cells were agglutinated at a titre of 1024. No agglutination occured when using red cells sensitized with Vi antigen against O antisera and O antigen coated red cells were agglutinated by anti Vi to a titre of 32.

In a next series of experiments, the specificity of agglutination of the antigen coated red cells by its corresponding antibody was determined (Tabte I). The results revealed high titres of haemagglutination by homologous antibody (512-1024), while low titres (8-16) or absence of agglutination were obtained with heterologous antibody as shown in the table. It was also noted that red cells coated with S.typhi Vi antigen were only agglutinated by Vi antisera and that cross-reactions only occured between S.typhi and S.paratyphi A.B.C. to low titres (16 or less) and there were no cross-reactions between S.paratyphi A.B. and C indicating a high degree of specificity of the test.

Tabte II shows the titres of antibodies in typhoid cases which agglutinated red cells coated with S.typhi O antigen in

TABLE I — Specificity of the Haemagglutination Reaction

Antisera	Haemagglutination Titres				
	Cells coated with antigen				
	S. typhi Vi	S. typhi O	Para A O	Para B O	Para C O
S. typhi Vi	1024	32	—	—	—
S. typhi O		>1024	16	8	8
S. paratyphi A O		8	512	—	—
S. paratyphi B O		8	—	512	—
S. paratyphi C O		8	—	—	1024

TABLE II — Typhoid patients antibody levels estimated by Haemagglutination

Samples	No. of specimens tested	Haemagglutination Titres								
		8 or less	16	32	64	128	256	512	1024	>1024
First	99	17	7	12	9	16	17	16	4	1
Second	102	8	7	6	6	22	13	19	15	6
Third	99	5	7	8	9	15	14	16	13	12

the three samples obtained from patients. It is evident that high titres of S.typhi antibodies could be demonstrated by haemagglutination as 70 out of the 99 third samples showed titres of 128 or more; 65 of the second and 45 of the first samples also had similar titres. From these data it can be concluded that the haemagglutination reaction is valuable by its great sensitivity. Another finding was that only one case had a titre of above 1024 in the first sample, while 6 in the second and 12 in the third samples reached that titre.

By comparison of the titres in the three samples, it appeared that most of the cases gave a twofold, a fourfold or even greater rise in the consecutive haemagglutination titres.

As regards the paratyphoid A cases (Table III), it was found that most of the sera agglutinated the red cells coated with S.typhi O antigen at low titres not exceeding 64 and that only 9 out of the total 39 cases gave higher rising titres, this being most probably an indication of a S.typhi and not a S.paratyphi A infection.

TABLE III — Paratyphoid A patients antibody levels against S. typhi O

Samples	No. of specimens tested	Haemagglutination Titres								
		8 or less	16	32	64	128	256	512	1024	> 1024
First	34	10	9	8	2	1	—	2	—	2
Second	35	5	6	12	5	1	3	1	1	1
Third	32	6	7	6	9	1	3	3	1	1

Table IV shows the results of 53 sera from typhoid cases and 12 sera from paratyphoid A cases being tested for presence of VI antibodies by haemagglutination. Vi antibodies were only found in 8 first, 11 second and 16 third samples of the typhoid cases and in one second and two third samples of the paratyphoid A cases.

Vi titres in the typhoid cases were generally low except in one case, where in the first sample it was 16, rose to 256 in the second and reached 1024 in the third sample, probably indicating an infection with a S.typhi Vi strain. One of the paratyphoid A cases had also shown a high titre of S.typhi O antibody (512) indicating that most probably the infection had been caused by S.typhi.

TABLE IV — Level of S. typhi Vi antibodies in 53 typhoid and 12 paratyphoid cases

Samples		Haemagglutination titres									
		2	4	8	16	32	64	128	256	512	1024
Typhoid	First	2	3	2	1	—	—	—	—	—	—
	Second	2	3	1	1	—	2	1	1	—	—
	Third	1	6	4	2	1	1	—	—	—	1
Para A	Second	—	1	—	—	—	—	—	—	—	—
	Third	—	2	—	—	—	—	—	—	—	—

When the levels of S.typhi O antibody in the control group were evaluated in Tables V, it appeared that 36, out of the 65 had a titre of 8 or less and 15 a titre of 16 and that all the sera did not contain Vi antibody.

TABLE V — Antibody levels against S. typhi O by haemagglutination in controls

No. of controls	Haemagglutination titres							
	8 or less	16	32	64	128	256	512	1024
65	36	15	8	6	—	—	—	—

DISCUSSION

By he use of the haemagglutination technique described in this study, it was possible to establish a reliable and reproducible method for measuring antibodies against S.typhi in human sera. The test appeared to be sensitive and specific and generally correlated well with the clinical and other

bacteriological methods of diagnosis. The technique was applied to a relatively large number of cases and controls.

The antigen responsible for the sensitization and subsequent agglutination by corresponding antibody seems to be associated with the O antigen and is type specific for all organisms. Successful results were obtained in the experiments demonstrating the specificity, as cross-reactions only took place between S.typhi and S.paratyphi A.B. and C at low titres and none at all between the S.paratyahi A.B. and C.

From the analysis of the control serum antibody levels, it is suggested that a titre of 16 or less may be considered as an average S.typhi antibody level among the normal Egyptian population, evaluated by the haemagglutination technique. as 78,4% of this control group gave these titres. Sera with high titres and sera showing a significant rise from the normal titres should be considered as positive as many cases gave titres above 512. It would similarly be reasonable to consider a slight rise or a titre of 64 in the third sample to be due to a nonspecific increase in titre (Recall phenomenon).

Vi antibodies were found in 16 out of the 53 cases of typhoid fever examined (30.2%) and the titres were low. This low incidence of the Vi antibodies in the present series may be due to the fact that most infections were not due to a S.typhi Vi strain.

In the experiment where a heart killed 24 hr agar culure suspension was used instead of the O antigen, the results were similar; therefore the use of this suspension will greatly simplify the technique for routine diagnostic work.

The sensitivity and specificity as well as the simplicity and rapidity of the haemagglutination technique, place this test in the foreground of the diagnostic measures in enterica infection.

SUMMARY

The haemagglutination technique was applied to evaluate the antibodies against S.typhi O antigen in a series of three consecutive serum samples from 154 typhoid cases, 39 paratyphoid cases and 65 normal controls.

Similarly antibodies against S.typhi Vi antigen were determined by the same method in 53 typhoid and 12 paratyphoid cases as well as the controls.

Haemagglutination was found to be highly sensitive, type specific as well as simple and rapid to perform and is recommended to be used in routine laboratory diagnosis of S. typhi infections.

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PHAGE TYPING OF THE TYPHOID BACILLUS [1, 2]

By

PIERRE NICOLLE[3]

General Methods of Phage Typing

If one tests several cultures of the same bacterial species or of the same serotype by an appropriate bacteriophage, it is often noticed, particularly if these cultures have been isolated in different places, that some of them are very sensitive to the lytic action of that phage, whilst others are far less sensitive or even not at all. By means of a single bacteriophage one can thus schematically divide a bacterial species into two groups, sensitive strains and refractory strains.

If instead of a single bacteriophage, several are used, each one having its own particular spectrum of activity, it would be possible to increase the number of groups subdividing that species. Each of these groups is defined by a certain pattern of lytic reactions, different from the patterns obtained with other groups.

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1. The opinions expressed are those of the author and do not necessarily represent the views of the Navy Department or the Egyptian Ministry of Health.
2. Translated from the French.
3. Pasteur Institute, Paris, France.

Such subdivision of a bacterial species by a combination of bacteriophage is called phage typing (in French, «lysotypie»).

In certain instances, when the phage is only weakly lytic, one must use it undiluted. However it is preferable, when the titer of the phage allows it, to use it in dilution. The lytic activities are then more specific, especially when the phages possess a high degree of adaptability. The most remarkable example of phage typing using adapted and diluted phages is the method of Craigie for the typhoid bacillus. This method is carried out using numerous preparations adapted from a single bacteriophage, the Vi phage II.

The main phage typing systems now in use are those of the staphylococci; diphtheria bacilli ; mycobacteria ; *Brucella ; Klebsiella; Vibrio cholerae* and *V. El Tor;* enteropathogenic *Escherichia coli;* and especially, the various *Salmonella;* typhoid and paratyphoid bacilli, S. *typhimurium,* and a few others. In this presentation, I shall talk only about phage typing of the typhoid bacillus, which is of particular interest to the epidemiologists of Egypt.

Phage Typing of the Typhoid Bacillus

Phage typing of *Salmonella typhi* (typhoid bacillus or Eberth's bacillus) is a model of its kind. It is important to briefly recall the chain of events that led to development of this method.

In 1934, Arthur Felix, at the Lister Institute of London, discovered a new antigen of the typhoid bacillus. This antigen presented properties different from those of other antigens already known for the same bacillus. As it was always abundant in cultures of bacilli isolated from severe cases of typhoid fever and was more or less completely missing in benign cases. Felix considered it as the only, or at least, the main factor responsible for the virulence of the typhoid bacillus; hence the name Vi antigen that he gave it (Vi being the first two letters of the word virulence).

Shortly thereafter, in 1936, Craigie and Brandon in Canada, Scholtens in Holland, and Sertic and Boulgakow in France, one group independently from the other, ascertained that some bacteriophages act on most of the Vi positive strains *S. typhi*, but that they were always inactive against the Vi negative bacilli. From this they concluded that the Vi antigen must be the specific receptor or these particular phages and for this reason named them Vi phages.

In 1938, Craigie and Yen noticed that one of their Vi phages, Vi phage II, possessed exceptional powers of adaptation, Upon growing it on a not very sensitive but not completely refractory Vi positive strain of *S. typhi*, it became extremely active on that strain and in the same time on a few others, for istance those isolated in the same epidemic location. Its lytic activity did not undergo a significant rise for other strains coming from different locations. Therefore, the Vi phage II adapted to a Vi positive strain of *S. typhi* has, become, in some way, specific of a whole group of typhoid bacilli.

Carrying out in the same way the adaptation of the Vi phage II to a great number of *S. typhi* strains isolated in different locations and in various areas, these authors obtained a series of adapted preparations having distinctive properties. Each was specific for one variety of the typhoid bacillus. These varieties were called the Vi phage types of *S. typhi*. Thus, Craigie and Yen demonstrated the existence of several distinct and stable varieties of *S. typhi*, until then considered as one of the most homogeneous among bacterial species pathogenic for man. (Figure 1).

Thanks to the creation, by Craigie and Felix, of an International Committee for Enteric Phage Typing affiliated with the International Association of Microbiologic Societies and of an International Reference Laboratory entrusted with the preparation of the adapted phages and of their distribution to the members of the Committee and thanks also to the research of several of its members, the number of phage types

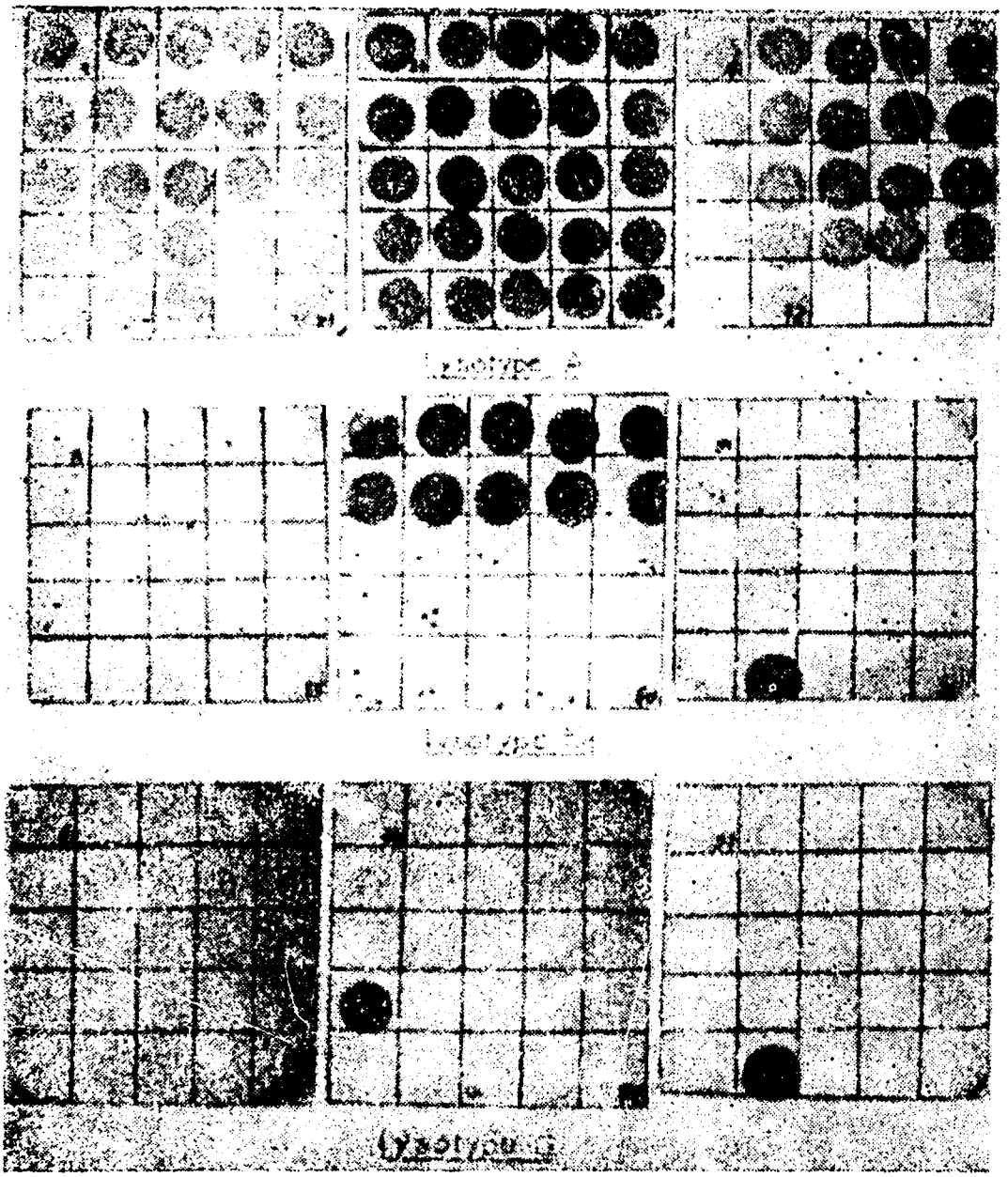

Figure 1: Phage Typing of Salmonella typhi, phage types A, E1, and G.

of *S. typhi* increased from 24 in 1946 to almost 90 today. (Table 1) (Table 2).

New Phage Types Under Study

To the 87 phage types recognized by the Central Reference Laboratory will be added a good dozen of unrecognized phage types discovered by the directors of Phage Typing Centers of various countries (particularly France, Japan, and Rumania) that are waiting for a response from the International Laboratory.

Untypable Typhoid Bacilli

A significant proportion of *S. typhi* strains, varying according to areas, escape phage type identification by the

TABLE 1 — The 88 Vi Phage Types of Salmonella typhi Recognised to Date (January 1970)

A	0	39
B1, B2, B3	T	40
C1, C2, C3, C4, C5, C6, C7, C8, C9, C10; C11	25	41
D1 D2, D4, D5, D6, D7; D8; D9; D10; D11	26	42
E1, E2, E3, E4, E5, E6 E7, E8 E9, E10	27	43
F1, F2, F3, F4, F5, F6, F7, F8	28	44
G1, G2, G3	29	45
H	32	46
J1, J2, J3, J4, J5	34	47
K1, K2, K3	33	48
L1, L2	36	49
M1, M2, M3, M4	37	50
N	38	

TABLE 2: The Vi Phage Types and Subtypes of S. typhi Discovered by the French Center for Enteric Phage Typing (Pasteur Institute)

1) Far-East (Vietnam, Cambodia):
E10, G3, J3, J5, M2, M3, M4,
and 5 other types under study

2) Spain and South America:
46, F8

3) Central Africa;
Central African variety of phage type C1

4) 9 subtypes of phage type A
11 subtypes of phage group I+IV

method of Craigie and Felix. These untypable strains may belong to one of the following three groups:

a) *Vi Negatve Strains:* We have seen that the Vi phages and more specifically the Vi phage II cannot act on strains not possessing Vi antigen. This antigenic factor may generally be lacking in strains from the same focus in certain benign epidemics as noted by Felix. However, often the lack of Vi antigen is due to an accident during isolation of the typhoid bacillus, when a Vi negative colony is selected among many other Vi positives. In order to avoid this risk, Felix advises choosing not just a single colony but a least six colonies and planting them in the same tube of broth.

If the majority of the bacteria in the culture is Vi negative, one can, with a bit of luck, manage to save it for phage typing by examining the colonies with oblique transillumination. Some appear more shiny and finely granular with an intense orange-yellow iridescence. These are usually the Vi positive colonies.

They are quite different from the less opaque and coarsely granular Vi negative colonies that surround them.

b) *Vi Positive Strains Not Susceptible to Any Form of Adapted Vi Phage II (Felix's Untypable Vi Strains or Group I+IV).*

Some Vi positive strains do not react with any preparations of the adapted Vi phage II, but they are generally susceptible to other Vi phages, in particular to Vi phage I, Vi phage IV, or both. For this reason, it was proposed to classify them in a temporary group, group I+IV, that probably contains some till now unrecognized phage types to which some day one may be able to adapt Vi phage II. This unmatched group renders epidemiologically the same services as a true phage type, especially if one adds the recommended biochemical and biological tests to the phage typing. (Figure 2).

c) *Vi Positive Strains Giving too Wide a Variety of Reactions to Interpret:*

Felix called these Vi degraded cultures. This designation tends to be confusing, because their Vi antigen is perfectly normal and not degraded, as one might think from this name. We have proposed the term « alienosensitive » cultures, that is to say slightly « foolish » in their sensitivity to the preparations of adapted Vi phage II. The alienosensitivity often results from degredation by progressive and irreversible acquisition of susceptibility to heterologous phages. A normal culture of phage type C1, for instance, will undergo confluent lysis by all the group C phages (from C1 to C11). Furthermore, it will display lesser, but still important, reactions with a great number of other phages. Growing old, such a culture could undergo an increase in sensitivity to these other phages. The reading thus becomes difficult or impossible. **(Figure 2).**

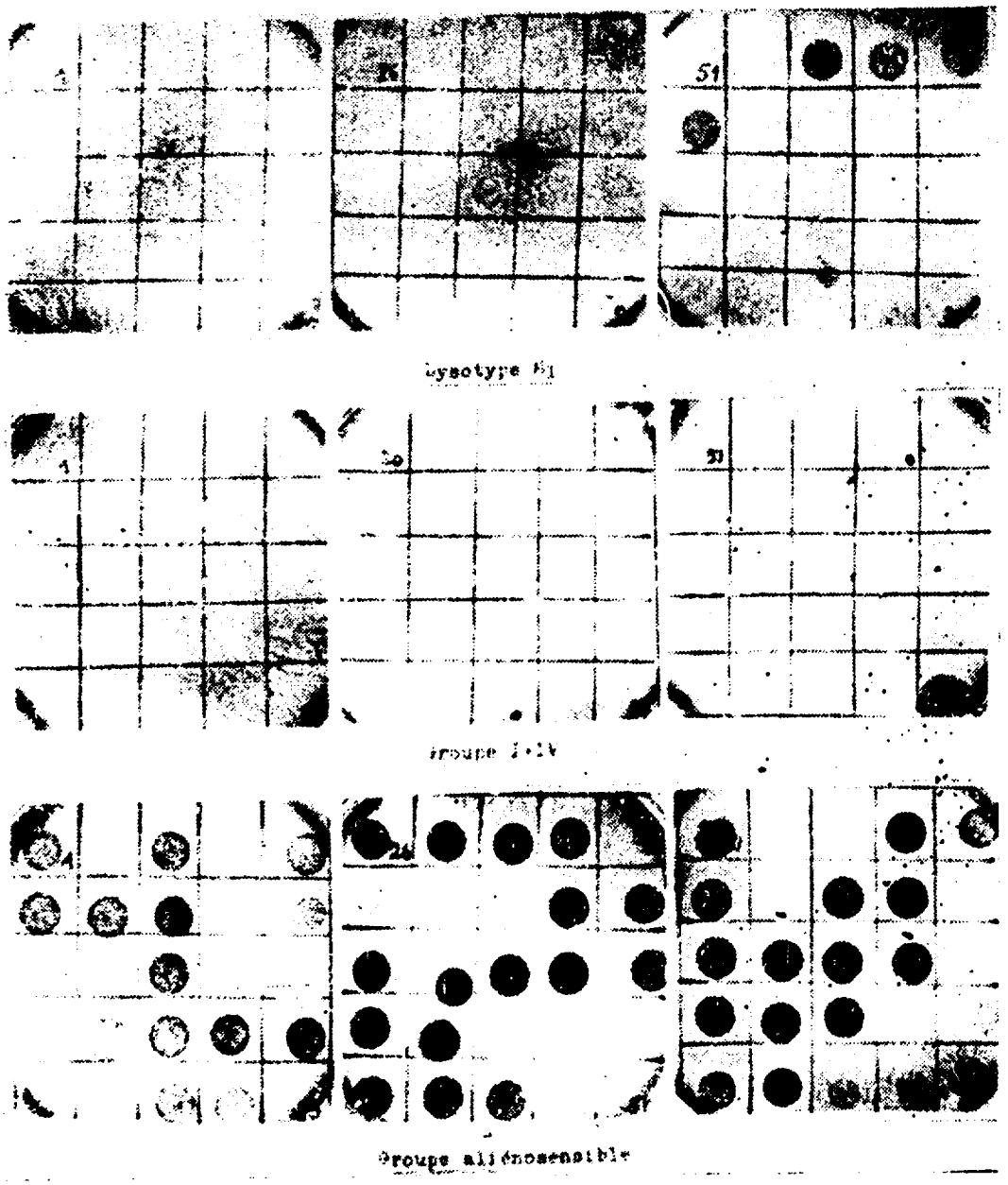

Figure 2: Phage Typing of Salmonella typhi. Phage Type M1, Non-typable Strains Termed <I - IV>, and Alienosensible

Technic Used in the Phage Typing of Salmonella typhi

Craigie's original method consisted of inoculating the bacteria onto an agar plate using a calibrated platinum loop making each droplet inoculum into a separate circular disk of one centimeter in diameter. As many circular disks were made as there were phages to test. Then, one desposited at the center of each inoculated disk, still using the platinum loop, a droplet of phage (a different phage for each disk) and spread it in a second disk concentric with the first one and slightly smaller. By this way there remained a margin of a few millimeters between the two disks where there was no contact between the culture and the phage. After overnight incubation, some disks did not show any trace of lysis (negative reaction). Others were sprinkled with more or less numerous plaques. Others presented a confluent lysis surrounded by a circle of normal culture (positive reaction).

This method, excellent in many aspects, presented a serious inconvenience. It required too much time. Time was saved by substituting the numerous disk inocula by platinum loop with a single inoculum covering the whole surface of agar plates. This was done either by depositing a few droplets of a young culture (less than 24 hours) in broth or peptone solution and spreading this with a sterile glass spreader or by covering the whole agar surface with several ml of culture broth and then removing excess liquid by means of mechanical suction consisting of a thin pipette connected to a Kitasato's vacuum bottle. Because one is dealing with pathogenic organisms, it is best to place 20 to 50 ml of an antiseptic solution (chlorine solution) in the bottle.

The inferior surface of the agar plates is divided into sixteen squares with glass marking ink. A drop of each phage is deposited in each square by means of a thin pipette, a tuberculin syringe, or an eye dropper fitted with a hypodermic needle.

Interpretation of Different Phage-typical Patterns of S. typhi.

If we examine the table of *S. typhi* Vi phage types, we see that certain phage types undergo confluent lysis with the homologous phage and that they d onot react or they react mildly, forming only rare plaques, with other phages. For example, this is the case with phage type C4 or phage type 37.

In another category of phage types, one would classify those that, in addition to confluent lysis with the homologous phage, give very strong reactions with several phages. Some are sensitive to all the phages that belong to the same group as the homologous phage. This is the case with the leading phage type of a group or a family. For instance, phage type C1 is sensitive not only to its specific C1 phage, but also to all the eleven other phages of the C family. This is also the case of phage type E1 which is sensitive to the ten phages of the E family and phage type M1 which is sensitive to the four phage of the M family.

Other phage types are sensitive not only to their homologous phage, but also to phages not belonging to the same family. For instance, the phage type 29 gives confluent lysis not only with phage 29, but also with multiple other phages. These are phage types of a less specific sensitivity. For their diagnosis, one should keep in mind not only the specific reaction with the homologous phages but also the reactions with the heterologous phages. In other words, in this case, it is not only the specific reaction that determines the phage type but the entire phage type pattern.

A third category of phage types includes those of group B. They undergo the confluent lysis with their homologous phage, but, in addition they give important reactions (numerous plaques or even almost confluent lysis) with several heterologous phages. The diagnosis of these phage types is often uncertain, with the exception of phage type B2 whose reactions are relatively easy to interpret and which is confirmed by always being xylose negative, at least for European strains.

Lastly, in a final category, we will place the phage type A which is fully sensitive to all the preparations adapted from the Vi phage II. When one has succeeded in adapting the Vi phage II to a new phage type, one can be almost sure that, at the routine test dilution for phagetyping, the newly adapted preparation will give confluent lysis not only on this new phage type but also on phage type A.

If all the phages produce confluent or almost confluent lysis, with the exception of two or three that give nothing or only plaques, we will call them « imperfect A cultures ». Let us also point out that certain phage types can progressively transform themselves in the media used for preservation, first into alienosensitive cultures, then to imperfect A cultures, and rarely into normal phage type A. It seems that the same transformation can occur also by mutation, or loss of type-determining prophage at least incertain cases (D1 transformed into A).

Stability of Phage Types

The value of phage typing method depends above all, of course, on the *in vivo* stability of the phage types and less important on the *in vitro* stability, that is to say in the culture media and in the preservation media

For *S. typhi* the *in vivo* stability, without being absolutely perfect, is satisfactory. The *in vitro* stability is relatively less reliable. Some culture lose their Vi antigen quite rapidly. Others undergo degradations in their phage type patterns. Nevertheless, thanks to certain precautions, it is often poseible to avoid these alterations. Thus, to avoid losing the Vi antigen, Dorset's media is used, as Felix recommended. Deep inoculation into agar is at least as good, but it is mainly by lyophilization of cultures as soon as possible after their isolation that one obtains the most remarkable results in the conservation of phage type patterns.

Geographic Variations of the Distribution of S. typhi Phage Types

Felix (1955) indicated two groups of phage types in his report on the distribution of phage types in different parts of the world. The first comprised the common phage types, the second the rare ones. We, too, have repeatedly pointed out the uneven distribution of different phage types according to geographic origins of the strains. Some are cosmopolitan phage types; some are common in Northern Africa; others are principally found in the Far-East, Australia, Peru, and Chile; while others seem to be of South American origin.

Significance of Phage Types of Typhoid Bacilli in Applied Epidemiology in the Study of Particular Foci.

What is the use of *S. typhi* phage typing, and, in general, what is the use of phage typing methods in applied epidemiology ? If a person suffers from typhoid fever or any other transmissible bacterial disease, or if he is germ carrier, then the bacilli that are isolated from this person during his illness or carrier state must necessarily belong to the same phage type, no matter what the source of the culture is (blood, stool, urine, bile, pus, cerebrospinal fluid, etc.) and no matter what treatment he has received (with the exception of treatment by bacteriophages, which may sometimes alter phage types.)

If this patient or this carrier infects persons in his surroundings, no matter what the number of these contacts is (small familial focus, small school epidemics, barracks epidemics, or large epidemics caused by the contamination of drinking water or food) the phage type will be the same for all; the patient, the carrier, and the infected persons. Hence it follows that, by means of phage typing, one can establish the relationship between cases, trace the original source of infection, point out the responsibility of a carrier for beginning a focus of infection, and having all the facts, be able to take the appropriate measures in the case of each focus or each epidemic and

thereby stopping its spread or even succeed in achieving the eradication of the illness in a given area.

Phage typing throws light on epidemiological research that would often, without it, remain confusing. One cannot seriously study an epidemic of typhod fever without using this method. It is therefore necessary that the germ be isolated before administering antibiotics. Laboratory directors should send the isolated cultures to the Phage Typing Center of their country, or if there is none in the country, to the Phage Typing Center of the Pasteur Institute, which will carry out the identifications free of charge.

Significance of Phage Typing of Typhoid Bacilli in the General Epidemiology of Typhoid Fever

The uneven geographic distribution of *S. typhi* phage types presents very interesting problems in the general epidemiology of an illness as cosmopolitan as typhoid fever. For instance, why is a phage-type such as E1 actually widely distributed all over the globe, whilst another like 37 has remained confined to relatively limited area (Asiatic South-East) ? Why again, does one find an exotic phage-type like the M1, which is very rare in the West, in two or three areas as far away from each other as the Far-East, Australia, and the west coast of South America ?

It appears as if the phage type A, that is sensitive to all preparations adapted from the Vi phage II and probably represents the primitive form of the typhoid bacillus, has diversified by the combined effect of mutations and of chance lysogenizations while evolving in different parts of the globe. It appears that some of its varieties, overflowing the limits of their initial domain no doubt owing to the intensity of human travel, have been spread a long way. For instance, Europeans, big travelers, must have scattered the most common phage types of their continent over the world (A and E1) and have given them a cosmopolitan character. Other varieties, less

favoured in this respect, have remained almost confined to the same localities where they first appeared. Phage typing of the typhoid bacillus can, in the long run, inform us on the movements of typhoid fever and on the sequence of events in the large epidemics in the world.

Limitations of Phage Typing and Possible Improvements:

Biochemical Subdivision of Phage Types

Among the proposed subdivisions we must first mention that of Kristensen. This author has recognized the existence of three biochemical types according to fermentation characteristics of *S. typhi* strains. De Blasi and Buogo have added a fourth. (Table 3)

Some phage types always or almost always consist of chemotype 1: C1, D1, E1, F1, G1, J1, L1, 37(etc. and others of chemotype II: B2 (except in Peru) M1, M2, M3, M4, 40, and 42.

In a third category are found biochemically mixed phage types: A, D6, N, O, T, 28, 29, group I+1V and group alienosensitive. These phage types have some strains belonging to chemotype I and others, in equal or almost equal number, to chemotype II.

The biochemical test thus performs two functions: Confirmation of the phage type for the phage types of uniform chemotype and also subdivision of biochemically mixed phage types.

Complementary Phage Types

Other subdivisions of some very frequent phage types have been proposed.. These are complementary phage types that allow recognition of 9 sub-types in the phage type A and 11 sub-groups in the group I+IV.

These complementary phage types just as phage types themselves, have a great application to epidemiology applied to the study of epidemic foci and to general epidemiology of typhoid fever. (Table 4)

Colicinogenic Property

Of great interest for basic research but with more limited practical interest to applied epidemiology, the research of the colicinogenic property permts the subdivision of *S. typhi* strains into two new varieties of very uneven numeric distribution: The non-colicinogenic variety takes in more than 99% of the examined cultures, the colicinogenic variety less than 1%. Certain phage types and the xylose negative variety of group I+IV always or at least often possess this antibiotic property: 10 (100%), 36 (50%), xylose negative variety of group I+IV (approx. 50%). Others only exceptionally have this property: E1, 38, positive xylose variety of group I+IV, alienosensitve xylose negative cultures. In spite of the rarity of the colicinogenic characteristic and even because of this rarity, when cultures of a same epidemic focus have it, it takes on great epidemiological importance which adds to those of phage typing and of biochemical differentiation.

SUMMARY

Phage typing enables us to recognize more than 80 definite and stable varieties of *Salmonella typhi*. It offers the epidemiologists a valuable means of detecting the origin of contagion, to follow its evolution and, knowing the cause, to aid in the fight against the spread of the epidemic and even to succeed in its eradication from a given area. The precision of the method is increased by adding to it Kristensen's biochemical test, the complementary phage typing, and examination for the colicinogenic property.

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PHAGE TYPING OF *SALMONELLA TYPHI* AND *S. PARATYPHI* A RELATED TO SALMONELLOSIS IN EGYPT[1]

By

RIFAAT HABLAS, PIERRE NICOLLE, and WARREN R. SANBORN

Strain identification of various bacterial species by the use of specific bacteriophages has been shown by numerous authors to be an important epidemiologic tool. Prime areas for application of this tool have been the study of *Salmonella typhi* types, introduced by Craigie and Yen (1938) and, more recently, *S. paratyphi* A by Banker (1955).

There have been several studies of the phage types of *S. typhi* and *S. paratyphi* A in Egypt employing these principles. One was done at NAMRU-3 about 20 years ago by Gillmore and Watts (1952). Another study was performed in Alexandria about 10 years ago by El Ghoroury, et al. (1961). The purposes of the

1. This study was supported by Bureau of Medicine and Surgery Project MR005.20.01.0261 A. The opinions and assertions contained herein are those of the authors and do not necessarily reflect the views of the Navy Department or the Naval Service at large.

present report are to compare phage type patterns of *S. typhi* and *S. paratyphi* A found today with those reported earlier and also to report several unusual results obtained from selected enteric fever patients.

MATERIALS AND METHODS

The majority of the *S. typhi* and *S. paratyphi* A strains for this study were obtained from acute enteric fever patients. These patients were part of a comparative treatment study conducted at the Abbassia Fever Hospital in Cairo. A few cultures were obtained from chronic salmonellosis patients on the NAMRU-3 ward. Most of these cultures were isolated from blood, but urine and stool isolates were also included. Except in certain instances, only one isolate per patient has been included in this report. All patients were residents of Cairo or nearby villages. There were no known epidemics or outbreaks included. Cases were not directly related as far as was known, but such a possibility was not absolutely excluded.

Salmonella cultures were isolated on Bismuth Sulfite Agar, Salmonella-Shigella Agar, or MacConkey Agar, both with and without preliminary enrichment in the case of urine and stool isolates. Blood culture methods were those presented elsewhere in this symposium by Sanborn and Dyer (1970).

Salmonella sp. were tentatively identified biochemically by reactions on Triple Sugar Iron Agar and a motility-mannitol medium. Presumptive positives were then further identified by «O» and «Vi» agglutination tests. Certain cultures of questionable identity were further studied by «H» agglutination tests.

Phage typing of *S. typhi* strains was performed by the basic method of Craigie and Felix (1947) with standard phages, as described by Nicolle et al. (1963). *S. paratyphi* A strains were phage typed by the method of Banker (1955). Further complementary phage typing of both species was performed, sub-groups of *S. typhi* phage type A by the method of Nicolle et al. (1963) and *S. paratyphi* A phage type 1 by the technique of Hablas and Nicolle (1969).

RESULTS

There were 189 strains of *S. typhi* selected for phage typing. All were «Vi» positive. Analysis of the phage typing patterns of this group revealed 20 phage types, including untypable strains and strains designated by Craigie and Felix as Vi degraded strains. (1+IV group of Nicolle et al., 1963). The distribution of the Vi phage types and their frequency of occurrence appears in Table I.

The five most frequently encountered Vi phage types were types generally considered to enjoy cosmopolitan distribution. These comprised 51% of the sample. If the untypable strains, 22.2%, and the I+IV strains, 7.4%, also cosmopolitan, were included, then commonly encountered phage types made up 80.6% of the sample.

The remaining 19.4% were those generally considered to be «exotic, «rare», «very rare» in world collections. Phage type 42 was considered to be «frequent» not «cosmopolitan», because it has been commonly found in North Africa but not elsewhere. Phage types designated «exotic» were those often encountered elsewhere in moderate numbers but which were not common in North Africa or the Middle-East. Phage types designated «rare» or «very rare» were those seldom reported by other workers in phage typing centers.

While phage type A was only 7.4% of this series, it has been generally considered one of the more common phage types found throughout the world. For this reason it was divided into subgroups by complementary phage typing. By this technique our strains fell into the three groups indicated in Table II.

The results of this study were compared with those of earlier studies. These comparisons formed the basis for Table III by including extrapolations of data from El Ghoroury et al. (1961) and Gillmore and Watts (1952). In general, each study yielded the same phage types, in-so-far as they were available at the time of each study. All phage types found in this study were available to El Ghoroury, but eight of the 24 phage types

TABLE I — Phage Types of Salmonella Typhi
(189 Egyptian Strains, 20 Phage Types)

Phage Types	Strains	Percent Found	Comments Frequency and Geographic Distribution
Untypable	42	22.2	Cosmopolitan
I+IV	14	7.4	Cosmopolitan
40	33	17.4	Cosmopolitan
C—1	22	11.4	Cosmopolitan
D—1	18	9.5	Cosmopolitan Rare in Far-East.
A	14	7.4	Cosmopolitan
E—1a	10	5.3	Cosmopolitan
42	9	4.8	Frequent in N. Africa. Rare in central Africa, Europe. and Far-East.
J—1	6	3.2	Exotic. Found in Africa and Far-East.
E—2	5	2.6	Exotic. Frequent in the Philippines. Very rare in Africa and Europe.
T	4	2.1	Exotic. Found in Middle East and Far-East.
C—5	2	1.1	Rare
E—1b	2	1.1	Very rare, Found in limited areas of Germany and Vietnam.
G—1	2	1.1	Exotic. Frequent in Congo and around Indian Ocean.
F—3	1	0.5	Rare
G	1	0.5	Exotic. Found in East Africa. India, and Far-East.
L—2	1	0.5	Very rare
0	1	0.5	Rare
36	1	0.5	Very rare. Possibly more common in S. America.
50	1	0.5	Very rare new phage type.

TABLE II

Complimentary Phage Typing

S. typhi Phage Type A

Type	Number
Tananarive	10
Montreal	3
Untypable	1

found by El Ghoroury or ourselves were not yet discovered at the time of Gillmore's study. Furthermore, Gillmore's phage types E-1 and G necessarily included the more modern sub-groups of these phage types E-1a, E-1b, G, and G-1. Certain rare phage types were found exclusively by each group of investigators. Three types were found exclusively by Gillmore; D-4, D-6, and N. Three others were found only by El Ghoroury: 27, 28, and L-1, and our study yielded F-3, 0, 36, and 50, phage types not found in either of the earlier reports.

Phage typing of the 88 *S. paratyphi* A strains yielded the three groups in Table IV. None of these were the rare phage types of this species. Phage type 1, a type considered to be of cosmopolitan distribution, further subdivided by complimentary phage typing, placed 91% of these 35 strains into subtype 1a, 3% into 1b, and 6% into 1c.

In the original group of more than 300 strains submitted to phage typing, there were multiple cultures from certain patients. Some of these were from different types of specimens obtained on a given day, while in other instances, these were later isolates from patients suffering relapse or periodic isolates from chronic carriers. Multiple cultures from most of these patients always yielded isolates of the same phage types. However, five of these patients yielded *Salmonella* strains of different phage types, shown in Table V. These results were confirmed in repeated tests. All of these cultures were from different specimens taken from each patient. In the case of the *S. paratyphi* A infections,

TABLE III — Comparison of Salmonella typhi Phage Type Studies in Egypt

Year :	1969	1958	1949
Author :	Hablas	El Ghoroury	Gilmore
Area :	(Cairo)	(Alexandria)	(Cairo)
Phage Types Strains :	(189)	(184)	(82)
Untypable strains	22%	17%	27%
Unadapted phages*	7	23	X**
40	16	8	X
C—1	12	13	22
D—1	10	<1	22
A	7	21	6
E—1a and E—1b	6	1	2
42	5	1	X
J—1	3	0	1
E—2	3	<1	0 0
T	2	4	2
G and G—1	1.6	4.9	9.8
C—5	1	<1	X
F—3	<1	0.0	0.0
L 2	<1	1	0 0
0	<1	0.0	0.0
36	<1	0.0	X
50	<1	0.0	X
27	0.0	2	X
28	0.0	1	X
L—1	0.0	<1	0.0
D—4	0.0	0 0	2
D—6	0.0	0.0	2
N	0.0	0.0	2

* Unadapted phages = I - IV of Nicolle and DVS of Anderson.

** X = Phage not available to Gilmore

TABLE IV

Salmonella paratyphi A Phage Types
(88 Egyptian Strains)

Phage Types	Percent Found	Expected Distribution
2	45	Frequent
1	36	Cosmopolitan
4	18	Frequent

TABLE V

Patients Carrying Multiple Phage Types
Salmonella typhi

Patient Number	Specimen Day or Date	Specimen Source	Phage Type
T—96	1	Blood	D—1
	24	Stool	Untypable
T—1088	2	Stool	D—1
	22	Blood	40
T—1010	24	Urine	E—2
	29	Blood	C—1

Salmonella paratyphi A

2144	30 Sep. 1968	Urine	1 (a)
	Same	Blood	2 (a)
2198	10 Mar. 1968	Urine	4 (b)
	Same	Blood	1 (b)

specimens were obtained on the same day, while with the *S. typhi* patients, the specimens were received on different days. From patients designated T-96 and T-1088 the specimens were taken before and after apparantly unsuccessful oral treatment with chloramphenicol. In the case of patient T-1010, both specimens were obtained subsequent to relapse following oral chloramphenicol therapy.

DISCUSSION

The geographic distribution and frequency of *S. typhi* phage types has been clearly described by Nicolle et al. (1964). Approximately 81% of our *S. typhi* strains tested were phage types considered to have cosmopolitan distribution throughout the world. The other 19% were less common or rare forms. Type 42 has frequently been reported from North Africa and might, therefore, be expected in this collection. Types J-1, T, E-2, and G were considered to be exotic. They have been infrequently found in the Middle-East, central or Eastern Africa, and the far Orient. Others were types rarely found anywhere. A few have been very rarely reported, for example E-1b which has previously been reported only from limited areas of Germany by Brandis (1955) and Vietnam. These results seemed to suggest that Egypt had become a «crossroads» for *S. typhi* strains. This situation may have derived from the geographic position of Egypt between Europe and the Orient, Egypt's commerce on the Nile, and the international community that populates Cairo.

Of *S. paratyphi* A strains typed, all were relatively common forms. None of the rare types 3, 5, and 6 were encountered In view of the various rare phage types of *S. typhi* that were found, it seemed unusual not to find a few of the rare *S. paratyphi* A strains.

When evaluating these recent phage typing studies with those studies done earlier, caution must be exercised when comparing percentages of the types reported, primarily because small epidemics or outbreaks could easily bias the picture. However,

there were several interesting relationships that became apparent in Table III.

The ratio of untypable strains in each study was relatively the same. Many new phage types were discovered in the 20 years following Gillmore's study. Since two of these, 40 and 42, were quite common in the present study, one might have expected to see the 27% untypables reported by Gillmore to be reduced further than only to the 22% that we found. Types 40, 42, and the unadapted phages together most probably comprised a large portion of Gillmore's untypable strains. Therefore, it would seem that the untypable strains found in the present study might contain a large proportion of newer phage types that could be unique to Egypt or this part of the world.

The prevalence of certain phage types remained relatively constant from one study to the next, for example; 40, C-1, and the untypable strains. On the other hand there were certain notable discrepancies. Type D-1 was frequently found in both Cairo studies, 20 years apart, while only one such strain was found in Alexandria. This might indicate a basic difference in the sources of typhoid cases in the two areas. Type A frequency was about the same in the two Cairo studies but was three times more frequent in Alexandria. The Alexandria results might indicate a different basic source of cases or might represent a small thyphoid fever outbreak at the time of the study. The E-1 phage type was more common in the present study than in the others. This, too, might be the result of a small outbreak of related cases. A gradual reduction in strains of phage type G over the years was also noted.

The presence of more than one strain of *S. typhi* or *S. paratyphi* A in a single patient, as indicated by differing phage type, was noted in five instances. Regarding the typhoid fever cases, several things seemed to be responsible for these results. PatientT-96 experienced a relapse. The second strain of *S. typhi* obtained from his stool was untypable and probably represented a Vi degradation of this strain. In patient T-1088, the two most likely explanations would seem to be either double infections or re-infection. Infection simultaneously with two strains of *S.*

typhi would seem to be the most likely answer in patient T-1010, where the specimens were obtained within a few days of each other. Transformation of Vi phage type in vivo was considered possible, but only for a few types. It seemed not probable in these instances.

Two dual *S. paratyphi* A infections were also noted. In each instance the specimens were obtained from the patient on the same day. Here, double infection seemed to be the most likely explanation. However, a second intriguing possibility suggested itself. It was noted that the complementary phage type of Hablas and Nicolle (1969) was the same in each pair of these isolates. It might be that these lysogenic phages used for complementary phage typing were actually more specific than the adapted phages of Banker normally used for phage typing of *S. paratyphi* A.

SUMMARY

Phage typing was performed on 189 strains of *Salmonella typhi* and 88 strains of *S. paratyphi* A obtained from enteric fever patients in Cairo, Egypt. There were 19 phage types and a group of untypable strains found among the *S. typhi* isolates. Three phage types of *S. paratyphi* A were identified. These results were compared with those of two phage typing studies done in Egypt 12 years and 21 years previously. Five patients apparently carrying dual infections of *S. typhi* or *S. paratyphi* A were discovered. The possible causes of this phenomenon were discussed.

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RELATION OF SCHISTOSOMIASIS TO TYPHOID FEVER

By

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Recent studies in our country have proved that there is an intimate relation between the development of the chronic urinary enteric carrier state and the urinary schistosomal infection. It was also observed that enteric infection in schistosomal patients usually assumes a characteristic form.

As regards the carrier state, it was first stated that a relation existed, between the incidence of the urinary enteric carriers and the wide spread urinary schistosomal infection among the Egyptian rural population. The recorded incidence of the urinary enteric carriers in our rural districts averages between 1 and 3 per cent of the whole population as shown by different investigators, Weir et al. 1952, Miller and Floyd, 1954.

Most surveys carried out in the Egyptian villages, have also demonstrated the higher incidence of urinary as compared to the faecal carriers, Neva 1949, Miller 1950, Weir 1952, Mohieldin 1962, Hathout *et al.* 1966. Reports from other countries than Egypt, put the ratio of urinary to faecal carriers as 1: 10-12. Browing, 1933 gives, figures from a central enteric depot in Eng-

land during the first World War, which show, only one urinary and 15 faecal carriers, out of 546 typhoid convalescents; one urinary and 23 faecal carriers, out of 837 cases of paratyphoid A; and one urinary and 23 faecal carriers out of 1425 cases of paratyphoid B. Donle, 1944, stated that no urinary carriers could be detected among 121 patients during an epidemic of paratyphoid A which occured in Vienna 1939. A similar investigation was carried out in Cairo by Neva 1949, out of 45 typhoid and 31 paratyphoid A patients, 2 typhoid and 7 paratyphoid patients developped the carrier state. All of his carriers were urinary all had urinary schistosomiasis and only one temporary faecal carrier was detected in his series.

Mohieldin 1962, found the precentage of urinary carriers to be 2.9, in a village near Cairo, with urinary schistosomal infection rate of 60 per cent. The urinary carrier rate of a subgroup of the same village, working in a factory where they were continuously investigated and treated for schistosomiasis, was 1.2 per cent only (the urinary schistosomal infection in this subgroup was 8.8 per cent).

The high incidence of the enteric urinary carriers in our rural areas, is generally believed to be due to the widespread schistosomal infection among the rural population. (Neva 1949, Halawani and Badran 1958). It was thought that the schistosomal lesions of the urinary tract paved the way to secondary infections by various organisms, including the enteric bacilli which established themselves in the different parts of the mucous membrane especially in the bladder and the pelvis of the kidney. (Morten 1949, Neva 1949, Walton 1949).

Recently our study on the enteric chronic urinary carriers among Egyptians, (Hathout *et al* 1966) shows that obstructive lesions of the urinary tract caused by fibrotic schistosomal lesions, act as a predisposing factor for the persistence of the urinary tract infection with the specific organisms, thus establishing the chronic enteric urinary carrier state.

However, it was obvious that urinary schistosomal infection in patients not suffering from urinary obstructive lesions

did not seem to be a predisposing cause for the development of the chronic urinary carrier condition.

Table I, shows that of 54 patients with urinary schistosomiasis, 64.9 per cent were passing enteric organisms in their urine 3 months after the beginning of convalescence, while 48.9 per cent and 46.9 per cent were still passing the specific organisms after 6 months and one year respectively. It is noteworthy that in the non-schistosomal group of 21 patients, not one was found who was passing enteric organisms in the urine.

TABLE I — Incidence of the urinary carrier stare 3, 6, 9, and 12 months after enteric infection.

Months after infection	Schistosomal patients				Non-Schistosomal patients	
	No. excluded	No. tested	Nl. still positive	% pos.	No. tested	No. pos.
3	0	54	35	64.9	21	0
6	5	49	24	48.9	21	0
9	5	49	23	46.9	21	0
12	5	49	23	46.9	21	0

Table II, clearly illustrates that in chronic enteric carriers, there is a remarkably high incidence of obstructive lesions of the urinary tract. Of 21 patients only 2 did not show such lesions on radiological examination. On the other hand, a low incidence of obstructive lesions is associated with freedom from the carrier state after enteric infection. In 11 non-schistosomal cases examined radiologically, no obstructive lesions were found, and none of the 21 non-schistosomal patients studied developped the carrier state.

TABLE II — Incidence of radiological ogstructive lesions in relation to the urinary carrier state

	Total no. patients radiologically exam.	No. with radiological obstructive lesion	%
Carriers in schistosomal patients (3 months after infection)	30	26	83.3
Carriers in schistosomal patients (12 months after infection)	21	19	90.5
Non-carriers in schistosomal patients	18	5	28.0
Non-carriers in non-schistosomal patients	11	0	0.0

Among the 25 patients who had evidence of urinary tract obstructive lesions, 22 patients had stricture producing dilatation of one or both ureters, one had diverticulum of the urinary bladder, and two had renal calculi, Fig. 1 and 2.

Cystoscopic examination of nine urinary carriers revealed the following :

1) All patients showed heavy schistosomal infiltration of the bladder. Sandy patches, small superficial ulcers, submucosal calcified masses, schistosomal nodules and severe cystitis were observed separately or in different combinations in all cases.

2) Narrowing of one ureteric orifice was seen in one case, and of both ureteric orifices in 7 cases. The remaining case had ureteric orifices of good size but they were deformed and surrounded by sandy patches and the right kidney showed a stone

Figure 1: Bilateral hydronephrosis and hydroureters, with bilateral ureteric strictures at lower end, in 15_year_old male chronic urinary carrier.

Fig. 2: Bilateral tortuous hydroure_ters, with evident stricture of lower ends of ureters, in 12 — year — old male chronic urinary carrier.

in its lower pole in the plain film. A catheter could be passed 24 cm into the right ureter, but only 15 cm into the left ureter where it met an obstruction.

In our series of the chronic urinary carriers, it was quite clear that the incidence of the urinary carrier state was much higher in children and young adults than in older ages. This can be readily explained by the higher incidence of schistosomal infection in these age groups. Table III.

TABLE III — Age incidence of chronic urinary carriers

Age (Years)	No. cases	No. carriers	No. cases lost to follow up
10—20	31	15	1
21—30	15	5	2
31—40	6	3	—
41—50	2	—	—

On the basis of the existence of an intimate relationship between obstructive schistosomal lesions of the urinary tract, and the development of the urinary enteric carrier condition, treatment would primarly necessitate correction of the obstructive lesion. To this end, antischistosomal treatment might prove useful in a number of cases but other cases would require some form of surgical intervention.

The danger of the enteric urinary carriers is expected to increase, particularly after the increase of the number of factories which are being constructed in the different parts of the country and to which workers from the rural population will have to move. Consequently, the number of enteric carriers may increase in the urban areas and will be definite danger when employed as food handlers.

Chronic salmonellosis complicated by schistosomiasis, has been reported from Egypt for Schistosoma haematobium infection (Hathout 1960, 1967) and from Brazil for Schistosoma mansoni infection (Nerves *et al.* 1967, 1969).

In Egypt, it has long been noticed at Abbassia Fever Hospital, that in some cases of hepato splenomegaly, there is prolonged fever without an apparent cause. As these patients were found to have schistosomal infection, usually with Schistosoma haematobium, the pyrexia was attributed to the so called «schistosomal fever». This belief was further supported by the clinical observation that after antibilharzial treatment of these patients, the fever subsided in a good proportion of them. This occurred before the discovery of chloramphenicol. Later when this drug was given to some of these patients, relapses occurred despite readministration of the drug as shown in the following figures. Fig (3) and Fig. (4).

Our clinical and laboratory findings have demonstrated that enterica in the Schistosoma hematobium patients assumes mainly a urinary, rather than an intestinal form. The clinical picture simulates that of other urinary infections. Chills which are common in these patients are probably due to invasion of the blood circulation by the enteric organisms which are intermittently shed off from this persistent focus of enteric infection. Chronicity of the disease is undoubtedly due to the damage, and urinary obstructive lesions induced by the urinary schistosomiasis.

This view is supported by the frequent isolation of enteric organisms from both the blood and urine of these patients on repeated cultures. The ready accessibility of the drug to the organisms in the urine and the superficial epithelial layers of the urinary tract, may explain the rapid response of the fever to chloramphenicol therapy as shown in the following figures (5, 6, 7, 8).

The lack of severe illness and intestinal complication in these patients is explained by the naturally acquired immunity produced by their frequent exposure to subclinical infection

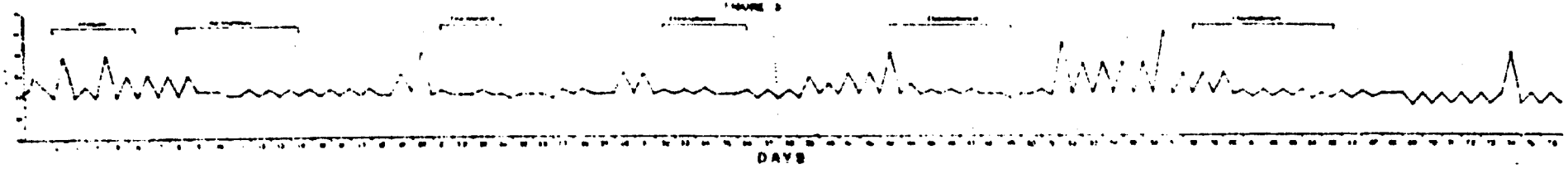


Fig. 3: Course of fever in a patient with paratyphoid A infection and haematobium schistosomiasis.

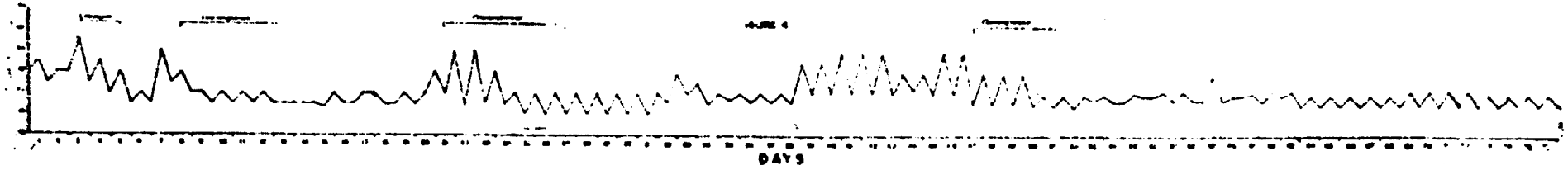
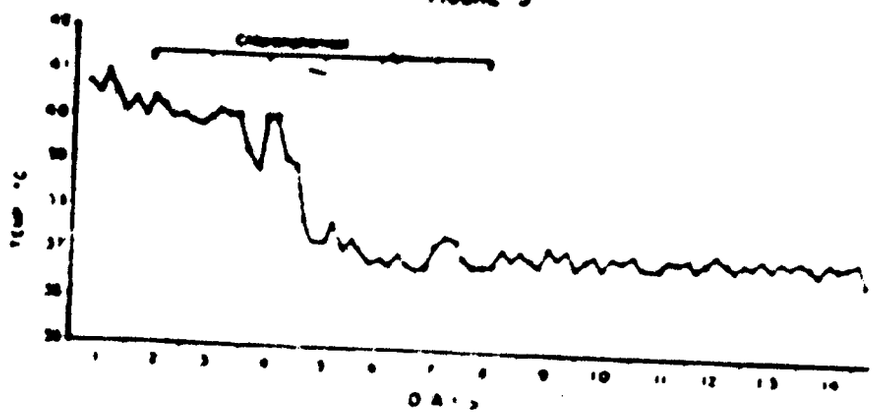


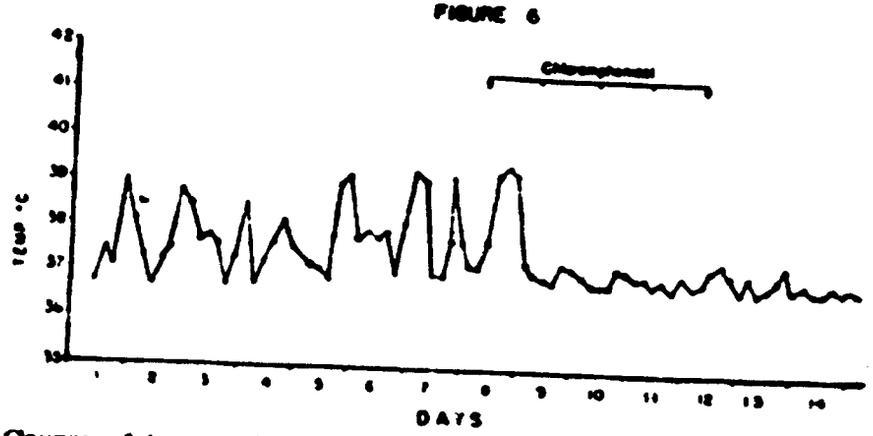
Fig. 4: Course of fever in a patent with paratyphoid A infection and haematobium schistosomiasis, with a history of fever for 90 days before admission.

FIGURE 5

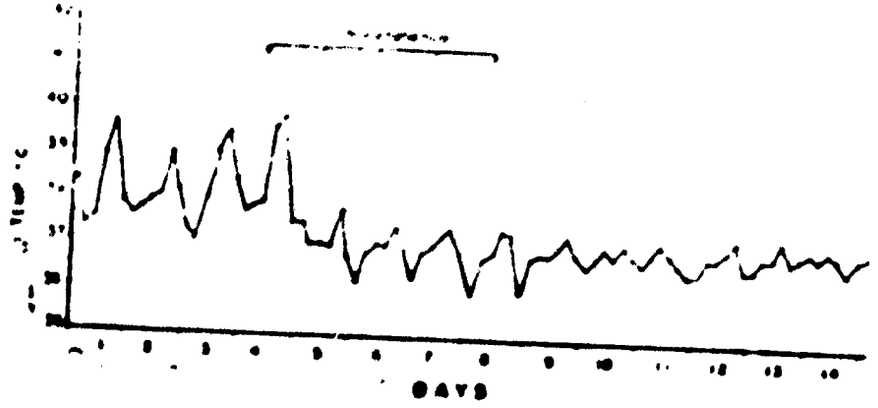


Course of temperature in a patient with typhoid fever, but without schistosomiasis, treated with chloramphenicol.

FIGURE 6

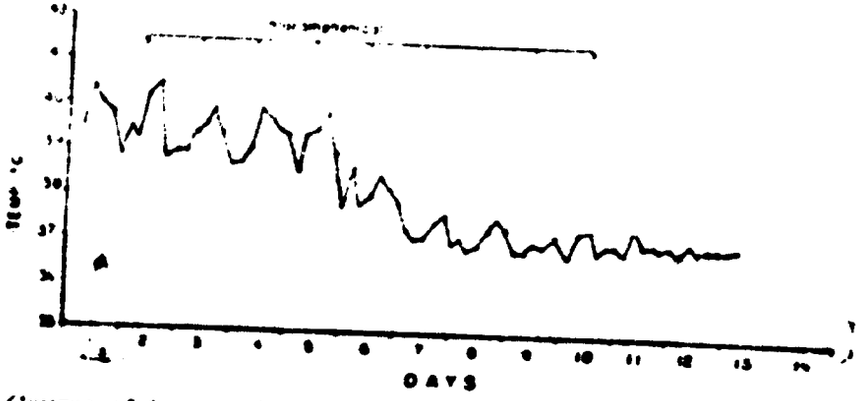


Course of temperature in a patient with paratyphoid A and schistosomiasis. Note rapid (within 24 hours) defervescence of fever after chloramphenicol therapy.



Course of temperature in a patient with typhoid fever and schistosomiasis, showing rapid defervescence of fever after chloramphenicol therapy.

FIGURE 8



Course of temperature in a patient with paratyphoid A infection, but without schistosomiasis; response to chloramphenicol therapy is gradual.

under their environmental conditions. It also explains the presence of enteric urinary carriers who do not show any clinical manifestations as stated by Miller 1950, who in a general survey of a Delta village, found that more than one third of the urinary carriers gave no history of fever. He also demonstrated a rising Widal titer in some of these enteric urinary carriers while they were afebrile, a further evidence in support of the enteric urinary tract infection. More over, Nayler and Caldwell 1953, in the Suez canal zone, examined the urine and sera from healthy British soldiers, and from Egyptians, suffering from urinary bilharziasis with and without the chronic enteric urinary carrier state, for agglutinins of enteric organisms both in the urine and blood. Their results gave evidence of liberation of antibodies at or close to the site of infection in the urinary tract of the chronic carriers.

There is another group of chronic salmonellosis, which has been described by Neves from BRAZIL, where Schistosoma haematobium infection does not exist. It occurs in patients with Schistosoma mansoni infection complicated by hepatic fibrosis. Neves stated that septicaemia was a striking feature. His pa- showed dramatic response to chloramphenicol as well as to antischistosomal therapy, the cure not being followed by relapses. Neves stated that the disease exhibited the clinical characteristics of reticulo endotheliosis very closely related to kala-azar. In fact, we have seen few of these cases among our patients with Schistosoma mansoni infection. Mechanism of prolongation of fever in these schistosomal patients with concomitant hepatic fibrosis may be attributed to the following:

1. The normal livers, have a filtering effect on bacteria circulating in the blood, as stated by Beeson and co-workers 1954. This function is impaired in cirrhotic livers, as well as in cases with schistosomal hepatic infiltrations.

2. Changes in hepatic circulation have been studied by Mann and other investigators, 1953, who demonstrated that anastomosis existed between portal and hepatic circulation. In Egypt, Hashem 1947, reported the presence of dilated capillaries

and sometime formation of angiomata in the fibrosed portal tracts in cases of bilharzial hepatic fibrosis. They may cause appreciable shunting of the portal circulation to the hepatic veins in the absence of clinically detected collateral circulation. Therefore, it is apparent, that, the loss of the defensive mechanism of the liver can be attributed, in part to intrahepatic communication between the portal and hepatic veins.

3. Schistosomal patients may have diminished production of antibodies as a result of the combined effect of malnutrition, anemia and other parasitic infections.

In conclusion, it is evident that measures for the control of schistosomal infection in the community do constitute a major procedure in the prophylaxis of the chronic carrier condition and accordingly in the incidence of acute enteric infections.

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CHRONIC URINARY SALMONELLA CARRIERS WITH INTERMITTENT BACTERAEMIA

By

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Mr. Chairman, Ladies and Gentlemen:

Today I would like to highlight some pertinent points on a subject that has already been discussed by several speakers at this meeting. This is the subject of chronic salmonellosis or prolonged salmonella septecaemia or bacteraemia or what I would prefer to call «chronic urinary salmonella carriers with intermittent bacteraemia». Call it what you like, the important point is to be aware of this relatively new entity — patients with typhoid or paratyphoid fevers which may last for months and months.

Watson (1) from South Africa first described the persistance for prolonged periods of S. typhi in some focus in the human body with intermittent invasion of the blood stream leading to a salmonella bacteraemia without the classic picture of typhoid fever. Neva (2) from Brazil described the clinical picture of what he termed chronic salmonellosis. His patients were all infected with S. mansoni, all had advanced hepatosplenomegaly and all were malnourished and anaemic. These patients had recurrent febrile attacks that lasted for months with salmonella organisms

cultured from the blood and occasionally from the stools, rarely from urine.

Chronic Salmonella carriers in Egypt are mainly urinary. Halawani, Abdalla, and Badran (3); and Hathout (4) and his group from the Abbassia Fever Hospital have shown that this condition is related to the damaged urinary tracts caused by S. haematobium infection and Hathout et al. (5) were the first to describe the clinical picture of salmonellosis complicating schistosomiasis in Egypt

Watson (1), Foster (6), Bokkenheuser (7) and others (8) suggested that it would be of great interest to hospitalize known urinary salmonella carriers and to carry out repeated blood cultures in order to determine whether these patients may occasionally also shed the organism into the blood stream leading to a symptomatic or asymptomatic bacteraemia.

During the past 5 years working with the Abbassia Fever Hospital group we succeeded in studying over 40 chronic urinary enteric carriers. Fifteen of these were followed-up in hospital for over 12 months and it soon became evident that these patients not only excrete the organisms in the urine but periodically over months will also shed them in the blood. These recurrent bacteraemic phases were not necessarily accompanied by fever and did not clinically resemble typhoid fever. This paper reviews our findings in these 15 patients (9).

All fifteen patients were young male farmers aged 8 to 29 years. All had haematuria and dysuria of from 3 to 15 years duration and all except 4 had received previous antischistosomal treatment. However, on admission to hospital 12 had active urinary tract infection with S. haematobium and intravenous pyelography showed that all 15 patients had damaged urinary tracts.

The presenting symptoms in the 15 patients were those of anaemia — pallor, dyspnoea, weakness and palpitation on exertion. Indeed it was this particularly refractory iron deficiency anaemia that first attracted our attention to the possibility of a chronic infection being the underlying cause of this persistent

anaemia. These patients contrary to the hookworm or schistosomiasis patients with anaemia would not respond to continuous oral ferrous sulphate treatment.

Their general clinical condition was usually very poor and the majority were malnourished and debilitated. Fever was not a prominent sign and usually these patients were ambulant and unaware that they were febrile. Some of these patients would have a sudden rise in temperature that would last for 2 to 3 days and then settle down to a niggling 100 to 101°F. This is in marked contrast to the continuous type of temperature usually seen in acute typhoid or paratyphoid fevers. Advanced hepatosplenomegaly was present in only 2 patients.

The Widal tests were not markedly elevated. Indeed in 2 patients though the enteric organisms were continuously cultured from blood and urine the Widals were repeatedly negative. The total white blood count also differed from that usually seen in typhoid and paratyphoid fevers and instead of being leukopenic ranged from 7 to 12,000 per c.mm.

In all patients the urine bacterial counts were over 100,000 per ml. signifying a urinary tract infection. Repeated stool cultures in all patients were negative. Blood cultures obtained from these patients at different times during the observation period grew the same organism as was being excreted in the urine.

Following chloramphenicol or ampicillin treatment the urine and blood cultures became negative, the refractory anaemia was corrected and their general clinical condition greatly improved. Unfortunately the majority of these patients relapsed both clinically and bacteriologically a few weeks after completing antibiotic treatment. Details of treatment using combined antischistosomal drugs and antibiotics are the subject of a separate paper (10).

Diagnosis of these patients particularly if the physician is unaware of the entity is difficult since the clinical picture does not resemble typhoid or paratyphoid fever. To sum up therefore a history of urinary schistosomiasis or the presence of S. haema-

lobium eggs in the urine in a debilitated, sick, and anaemic patient not responding to oral ferrous sulphate after deworming should arouse suspicion and lead to an active search for salmonella organisms in the urine and blood. A urine bacterial count over 100,000 per ml. with a damaged urinary tract evident on intravenous pyelography practically confirms the diagnosis. Repeated blood cultures are then necessary to isolate the organisms from the blood. Finally we consider that these patients actually harbour the salmonella organisms in the urinary tracts from which intermittently they are shed intravascularly; treatment, therefore, should aim first at relieving the bilharzial obstruction or defect.

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PATHOGENESIS OF CHRONIC SALMONELLA BACTEREMIA

By

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Presented at Joint symposium of NAMRU-3 and Abbassia Fever Hospital on Typhoid Fever, 15 January 1970.

The condition which has been described in the previous papers by Drs. Hathout and Farid, and which we refer to as chronic Salmonella bacteremia, is of interest not only as a new and unusual complication of endemic enteric fever, but also as a model for the study of chronic Salmonella infection in man. Our understanding of the mechanisms responsible for this peculiar clinical situation is incomplete, and at present, all our thoughts of the pathogenesis of chronic *Salmonella typhi* or *para-typhi* bacteremia are conjecture. Even the relation between salmonellosis and schistosomiasis in Egypt[1,2] must be considered hypothetical, as association is difficult to prove when two diseases are quite separately endemic within the same geographic area of study. However, in spite of this difficulty, we do believe that the appearance of chronic Salmonella bacteremia is in some way related to the presence of schistosomiasis in

these patients. This conviction is strengthened by our own experiences[3,4] and by the reports from Brazil[5] of chronic Salmonella bacteremia complicating *S. mansoni* infection there. We will consider this association later in the discusion.

First, I would like to pose a general view of this bacterial infection, pointing out similarities or differences between this syndrome and other Salmonella infections, including classical typhoid. I will speak first of the infecting bacteria, and later of the human host.

INFECTING ORGANISMS

Several factors concerning the Salmonella bacterium require consideration, any one of which, if altered, might lead to variation in the severity or duration of infection produced.

The oral *dose* of *S. typhi* which usually causes acute typhoid fever in non-immunized adult volunteers is between 10^7 and 10^9 bacteria.[6] An occasional individual, when thus challenged, will develop bacteremia without signs or symptoms of typhoid. Challenge with lesser doses of typhoid bacillus has caused gastrointestinal and febrile disease in man, followed by excretion of organisms for variable periods of time. We might postulate that occasional challenge with relatively small doses of Salmonella bacteria could lead to bacteremia and localization of infection without clinical signs of typhoid-like illness, particularly if certain host-factors predisposed to this. In Egypt, it is probable that Salmonella are a prevalent food and water contaminant, and that sub-typhoidal inocula are ingested frequently.

The *virulence* of these organisms should also be considered. This is a complex subject, but several statements can be made with regard to the Salmonella. Classically, the presence of the Vi antigen has been associated with virulence.[7] However, bacteremia may be induced by oral challenge of men[6] or chimpanzees[8] with non-Vi *S. typhi*. Perhaps bacteremia with less virulent Salmonella leads to relative host tolerance and a chronic course. In several instances such degraded of non-Vi *S. typhi* have been cultured at NAMRU-3. The hypothesis of lesser

virulence might also explain the preponderance of *S. paratyphi A* over *S. typhi* in chronic bacteremia. As well, phage typing of the locally isolated Salmonella may identify particular types associated with chronic bacteremia.

Resistance or drug-fastness of locally isolated Salmonella has not, to date, been noted, but as we shall hear later in this symposium from Dr. Hook, this presents potential problems. At present it is doubtful that drug resistance is important in the development of chronic Salmonella bacteremia.

Persistence of Salmonella, however, appears to be a significant feature of chronic Salmonella bacteremia and bacteriuria, as well as relapsing typhoid fever. McDermott[9] has described microbial persistence as the ability of an organism to persist within the host by means of various mechanisms, and to survive despite host defenses or chemotherapy. For systemic Salmonella infections, which are presumably of the reticuloendothelial system, it appears that the intracellular localization of the bacteria allows for considerable protection. This is the usual explanation offered for relapse of typhoid fever, even after adequate chloramphenicol therapy.[10] However, *chronic* bacteremia is not ordinarily a sequela of typhoid, although the carrier condition may be. Bacterial persistence in the instance of chronic bacteremia seems to be even more firmly entrenched. Cure is not effected with multiple conventional courses of chloramphenicol, and patients may relapse after treatment with ampicillin for one month.[4] Reinfection in this setting is highly unlikely, and clearly the bacteria are able to withstand not only host immune mechanisms but prolonged bacteriacidal drug therapy as well. Factors affecting this situation possibly include other adaptive properties of bacteria, and one in particular, the formation of bacterial variants or L-forms, deserves attention.

In essence, an L-form is a bacterium which has lost all or part of its cell wall, and which remains viable only if dwelling in an environment which is relatively hyperosmotic.[11] If the milieu is not hypertonic the cell bursts, as it no longer has the protection of its cell wall against an unfavorable osmotic gradient.

under their environmental conditions. It also explains the presence of enteric urinary carriers who do not show any clinical manifestations as stated by Miller 1950, who in a general survey of a Delta village, found that more than one third of the urinary carriers gave no history of fever. He also demonstrated a rising Widal titer in some of these enteric urinary carriers while they were afebrile, a further evidence in support of the enteric urinary tract infection. More over, Nayler and Caldwell 1953, in the Suez canal zone, examined the urine and sera from healthy British soldiers, and from Egyptians, suffering from urinary bilharziasis with and without the chronic enteric urinary carrier state, for agglutinins of enteric organisms both in the urine and blood. Their results gave evidence of liberation of antibodies at or close to the site of infection in the urinary tract of the chronic carriers.

There is another group of chronic salmonellosis, which has been described by Neves from BRAZIL, where Schistosoma haematobium infection does not exist. It occurs in patients with Schistosoma mansoni infection complicated by hepatic fibrosis. Neves stated that septicaemia was a striking feature. His pa- showed dramatic response to chloramphenicol as well as to antischistosomal therapy, the cure not being followed by relapses. Neves stated that the disease exhibited the clinical characteristics of reticulo endotheliosis very closely related to kala-azar. In fact, we have seen few of these cases among our patients with Schistosoma mansoni infection. Mechanism of prolongation of fever in these schistosomal patients with concomitant hepatic fibrosis may be attributed to the following:

1. The normal livers, have a filtering effect on bacteria circulating in the blood, as stated by Beeson and co-workers 1954. This function is impaired in cirrhotic livers, as well as in cases with schistosomal hepatic infiltrations.

2. Changes in hepatic circulation have been studied by Mann and other investigators, 1953, who demonstrated that anastomosis existed between portal and hepatic circulation. In Egypt, Hashem 1947, reported the presence of dilated capillaries

and sometime formation of angiomata in the fibrosed portal tracts in cases of bilharzial hepatic fibrosis. They may cause appreciable shunting of the portal circulation to the hepatic veins in the absence of clinically detected collateral circulation. Therefore, it is apparent, that, the loss of the defensive mechanism of the liver can be attributed, in part to intrahepatic communication between the portal and hepatic veins.

3. Schistosomal patients may have diminished production of antibodies as a result of the combined effect of malnutrition, anemia and other parasitic infections.

In conclusion, it is evident that measures for the control of schistosomal infection in the community do constitute a major procedure in the prophylaxis of the chronic carrier condition and accordingly in the incidence of acute enteric infections.

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from blood, and allow for a state of chronic bacteremia. This might take the form of decreased or defective circulating or tissue-fixed antibody production, or some abnormality of the phagocytozing cell itself. An intense hyperglobulinemia accompanies chronic Salmonella bacteremia, with increase in all fractions of immunoglobulins (unpublished observation). However, similar increases in immunoglobulins are observed in chronic schistosomiasis, and because all of our patients have co-existent schistosomiasis, it is impossible to relate this hyperglobulinemia to immunity to either infection specifically. Moreover, increased globulins do not necessarily imply increase in normal or specific antibody production. Certain fractions of these globulins may, in fact, interfere with effective phagocytosis of bacteria.[19]

Also related to schistosomiasis is an intense proliferation of reticuloendothelial cells. This is felt to be a nonspecific accompaniment of a chronic infection, but the function of these cells has never been assessed. Again, mere proliferation does not assure increased or even normal phagocytic ability, and it may be that effective filtering of bacteria is reduced in the reticuloendothelial hyperplasia of schistosomiasis.

At this point it might be well to refer to the effects of certain other disease conditions upon the ability of the host to withstand invasion by Salmonella. Diseases which are commonly complicated by Salmonella infections include malaria, relapsing fever, bartoneliosis and sickle-cell anemia.[20] As pointed out by Dr. Hook and his associates,[21] all of these diseases are linked by their effect on the reticuloendothelial system — particularly regarding the presence of hemolysis and erythrophagocytosis. They have shown an increased morbidity and mortality among hemolytic mice challenged with Salmonella.[22] Presumably hemolysis interferes with the normal clearing action of the RES by an effect of competitive inhibition or by an inactivation of normal killing ability of the phagocytic cells.

Brucellosis is, perhaps, another infection with which Salmonella bacteremia is associated. Several cases of concomitant infection have been reported,[23] and we have seen several cases here in Egypt. Brucella, of course, like Salmonella, are

intracellular parasites, and the presence of one may stimulate the blood stream invasion of the other.

At this point, it is well to raise again the question of what association schistosomiasis has to the generation of chronic Salmonella bacteriuria and bacteremia. The conclusion[1] that schistosomal uropathy predisposes to the development of the chronic urinary carrier state seems fair. In this sense, the damaged urinary tract is similar to the diseased gallbladder of chronic gallbladder carriers. We also believe that renal infection is common among patients with Salmonella urinary tract infections. Indirect evidence of this is the frequent finding of decreased maximal urinary concentrating ability among these patients (unpublished observation). This particular impairment of renal function is associated with renal infection.[24] A renal focus of infection is probably established in these patients, and perhaps, chronic intermittent seeding of the blood stream occurs secondarily. If, in addition, host defence mechanisms are impaired as described previously, the stage is set for chronic septicemia and its sequelae. This may be, I believe, the case, but evidence for all of this is lacking.

To summarize, let me list some of the possible approaches to future work on this interesting syndrome.

Investigations of the infecting bacteria might include careful analysis for differences between these organisms and organisms known to cause classic typhoid and paratyphoid fever in Egypt. It seems that phage typing would be very valuable here, as would assessment of virulence.

Attempted culture of bacterial variants or L-forms from patients with chronic Salmonella infection, particularly at times when their disease seems quiescent, might reveal continued but inapparent infection of blood and urine with these forms. Such a finding would go a long way toward explaining the extraordinary persistence of infection in our patients.

Investigations in the host which may prove fruitful include inquiring into intactness of immunologic and phagocytic functions. These are very specialized, but in large part, the present

day approach to the pathogenesis of infections is assuming such lines of study.[17,19,21]

I must mention possible experiments with endotoxin in patients with chronic Salmonella bacteremia. Explanations of the role of endotoxin in acute bacterial diseases, including typhoid, have been elegantly offered by Dr. Hornick and his associates.[25] Assessment of the reactivity to endotoxin of patients who have chronic Salmonella bacteremia might lead us to an understanding of the tolerance of infection which many of these patients appear to demonstrate.

Perhaps most important, is the necessity to establish the site of persistence, multiplication and seeding of Salmonella within the host. We have presumed that this syndrome is essentially a urinary tract infection with chronic blood stream invasion, but somehow this does not fit our classic concept of Salmonella as intracellular parasites. This probably is not merely a urinary tract infection with pyelonephritis. Salmonella may be persisting elsewhere, among the hyperplastic reticuloendothelial cells of the schistosomal infected host.

One last hypothesis, and an intriguing one, concerns the recently reported observation[26] that bacteria can infect and persist in the gut of schistosome worms. Various bacteria administered to mice infested with schistosomes were found to localize in the schistosome gut. Antibiotic treatment given to the mice could not eradicate the bacterial infection of the worms. Perhaps the association between Salmonella bacteremia and schistosomiasis is even closer than we imagine, involving persistence of Salmonella within the schistosome worm.

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TREATMENT OF S. TYPHI — PARATYPHI A URINARY CARRIERS

By

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Miller (1950) and Weir et al. (1952) reported an incidence of 3 % of typhoid and paratyphoid carriers of the population of Egyptian villages, while Neva (1949) reported an incidence of 2-5 %.

From our previous work (Farid et al. 1970) it was shown that several urine and blood cultures are needed in some patients before diagnosing the carrier state. This means that the incidence of Salmonella carriers is probably higher than that reported previously. These carriers constitute a serious public health problem since they are shedding large quantities of Salmonella organisms in water and are constant potential sources of infection (Chadwick 1954). Thus, from the public health point of view the successful management of these carriers is highly important. This paper discusses our experience in the management of these patients.

Patients:

30 male Egyptian farmers aged 4 to 30 years were included in study. All were ill for over 12 months, 9 were urinary excretors of S. typhi and 21 of S. paratyphi A and all had repeatedly positive blood cultures for the same organisms. All were excreting live eggs for S. haematobium in the urine.

Laboratory Investigations:

These included complete blood counts, serum iron, iron binding capacity, liver function tests, routine urinalysis, stool examinations for ova and parasites, Widal Brucella agglutination titres, on admission and repeatedly during and after treatment. Blood and urine specimens were cultured at every fever spike and routinely twice weekly before, during treatment and through out the prolonged follow-up period. Stool cultures were performed before treatment and once weekly during and after treatment.

All patients were followed up for 6 to 36 months.

Blood cultures:

Blood specimens were cultured on Castaneda-type two phase bottles (Castaneda 1947) and on 10% Ox Bile (Kaye, 1966).

Urine Cultures:

Loopfulls of urine were plated directly onto Selective media. In addition equal amounts (5 ml.) of urine and Selenite Broth (Difco) were incubated for 24 hours before plating on the same selective media.

Stool Cultures:

Swab samples of stool specimens were plated on Selective media. The swabs were then placed in Selenite Broth and treated in the same manner as urine specimens.

Pyelography:

Plane X-ray films of the bladder region, intravenous pyelography, and micturating cystograms were performed on selected patients.

Treatment:

Other parasitic infections were first treated. Patients infected with hookworms were treated with bephenium hydroxynaphthoate (Alcopar). All patients were then started on oral ferrous sulphate tablets 300 mg 3 times daily, this was continued as long as possible and for at least 4 weeks period before specific trratment for Salmonella infection was started.

15 patients received chloramphenicol 50 mg per kg body weight per day for 14 days (Group I).

11 patients were given oral ampicillin at a dosage of 100 mg/kg body weight per day for 4 weeks combined with antischistosomal treatment. In addition 3 patients of the 1st group who had relapsed were retreated according to the 2nd plan i.e. a total of 14 patients were treated with ampicillin combined with or following bilharzial treatment (Group II).

8 of these 14 patients were treated with niridazole 25 mg per kg body weight per day for 6 days and 6 received antimony tartrate 0.5 grain per 15 kg body weight per dose for 12 doses given intravenously twice weekly.

4 patients were only treated with niridazole at the same standard dosage for 6 days. (Group III).

RESULTS

Treatment

In all patients there were no improvement in anemia before starting the antibacterial therapy. (Hb levels did not rise for more than 1 gm after 4-8 weeks of ferrous sulfate

treatment). Results of management of the carrier state are shown in table I.

Group I. 4 patients were urinary excretors of S. typhi and 11 of S. paratyphi A. All patients treated with chloramphenicol relapsed (14 within 3 weeks and one after 56 days of chloramphenicol).

6 of these relapsed patients were retreated with chloramphenicol but all relapsed again. Anemia was rapidly corrected with ferrous sulfate with a rise of more than 1 gm Hb/week after treating the infection.

Group II 4 patients were urinary excretors of S. typhi and 10 of S. paratyphi A.

Of the 14 patients treated simultaneously with antimony tartrate or niridazole and with oral ampicillin for 4 weeks, 9 were cured and 5 relapsed within the last 4 weeks following treatment.

3 of the 5 patients continued to excrete S. paratyphi in the urine, one excreted the organism in the urine and blood. The fifth patient continued to excrete S. typhi in the urine. Anemia was also corrected after getting rid of infection.

Results of intravenous pyelography are shown in table II.

Group III. One patient was urinary excretor of S. typhi and 3 were of S. paratyphi A. All 4 patients were in the follow-up period. Three patients had repeatedly negative blood and urine cultures for periods of 3 to 7 months. One patient relapsed and excreted S. paratyphi A in urine 10 days after completion of treatment. This patient had a deranged glomerular kidney function (low creatinine clearance) which was not improved after treatment.

TABLE I - Results of treating S. Typhi and S. Paratyphi A Carriers

Number of Patients	Drugs Used	Number Cured	Number Relapsed
4 S. typhi Group I 15 11 S. paratyphi A	Chloramphenicol (14 days)	0	15 10 Blood and Urine 5 Urine (within 3 weeks, one after 56 days),
4 S. typhi Group II 14 10 S. paratyphi A	Antischistosomal Treatment (8 Ambilhar & 6 Tartar Emetic) Ampicillin	9	1 Blood and Urine 5 4 Urine (within 4 weeks)
1 S. typhi Group III 4 3 S. paratyphi A	Niridazole (Ambilhar)	3	1 Urine

TABLE II Results of intravenous pyeiography and Micturating ystograms performed for 13 patients treated with ampicillin and antischistosomal treatment

Total No. of Patients	No. of Patients with Schistosomal Damage of Urinary Tracts	Bladder Calcification	Bladder Nodular Filling Defects.	Hydrouret. Hydronephrosis	Stricture of one or both ureters with poor dye excretion.	Vesico ureteric reflux
13	12	6	3	4	2	2

Side-effects:

No serious side-reactions were experienced with either chloramphenicol or ampicillin. Both antibiotics were effective in controlling the bacteremia. Within a few days of starting treatment with either chloramphenicol or ampicillin all patients became afebrile and blood and urine cultures became negative for salmonella. As the general condition of the patients improved antischistosomal treatment could usually be started.

There were no side-effects with the use of antimony tartrate. Injections were given twice weekly and each intravenous injection was given slowly over a 10 minute period. With niridazole treatment, however, one patient had a profound-shock-like syndrome after the first dose and had to be treated with antimony tartrate. Of the 8 patients completing treatment with niridazole one had convulsions on the last day of treatment, one complained of a severe headache and 5 had marked nausoea and vomiting which was only controlled by reducing the total daily dose.

DISCUSSION

Although Miller 1954 and Halawani 1958 reported the successful treatment of their Salmonella carriers by a course of chloramphenicol, we failed to cure any of our carriers by a 14-day course of chloramphenicol. Our results confirm those reported by Douglas 1950, Woodward et al. 1950 and Topley 1955.

Halawani 1958 and Abdalla 1946 reported the higher incidence of urinary carriers in patients infected with urinary schistosomiasis. Hathout 1966 confirmed this relationship and reported a remarkably high incidence of obstructive lesions of urinary tract in those carriers.

Since we reported previously (Farid et al 1967, Farid et al. 1970) the resolution of soft tissue schistosomal obstructive

lesions in particularly young patients, with simple antischistosomal treatment, it was reasonable to think of combining antischistosomal treatment with prolonged antibiotic treatment in our plan to manage these chronic Salmonella excretors.

Because of the fear of bone marrow depression from prolonged course of chloramphenicol, ampicillin was considered a more preferable drug. Combined ampicillin antischistosomal treatment gave a good percentage of cure in these, since 9 out of 14 patients were cured, and their urine and blood cultures were repeatedly negative for the enteric organism for periods varying from 6 to 36 months.

The 5 patients who relapsed were those of permanent urinary tract damage (3 adults with no improvment of K.F.T. or obstructive lesions, and 2 young patients with vesico ureteric reflux).

Recently we thought of treating these patients with niridazole for 6 days to see if a simple antischistosomal treatment with the expected resolution of schistosomal obstructive lesions will cure these carriers. This will be a short term therapy and cheaper in price. Niridazole is preferable to other antischistosomal drugs as it was proved to have an in vitro bactericidal action against Salmonella organisms.

Of the 4 patients treated with niridazole tablets, 3 were cured, but the follow-up period is not enough to judge and the number of patients will be increased.

SUMMARY

30 male Egyptian farmers aged 4 to 30 years were studied. All were chronic urinary carriers of S. typhi or S. paratyphi A, and all were excreting live eggs of Schistosoma haematobium in their urine.

All patients treated with chloramphenicol for 14 days at a dosage of 50 mg kg body weight per day relapsed.

Patients treated with combined Ampicillin - antischistosomal treatment were cured with an exception of those with permanent damage of urinary tracts. Resolution of obstructive urinary tract lesions by antischistosomal treatment alone gives promising results.

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COMPARATIVE EFFICIENCY AND APPLICATION OF BLOOD CULTURE TECHNIQUES FOR ENTERIC FEVER DIAGNOSIS[1,2]

By

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and

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The use of ox bile in blood cultures for *Salmonella* was suggested at least as early as 65 years ago by Conradi (1906). More recently, Kaye et al. (1966) compared its efficiency with the common modern bacteriological medium Trypticase Soy Broth. They found more positive cultures by the ox bile method than in Trypticase Soy Broth cultures. During their experiments they noted that a disadvantage of broth blood culture was contamination, often introduced during the repeated sub-cultures requi-

1. This study was performed under project No. MR.02.01A of the Bureau of Medicine and Surgery. The opinions contained herein are those of the authors and do not necessarily represent the view of the Navy Department or the Naval Service at large.

2. The use of trade names is for identification purposes only and does not imply endorsement of any single product.

red to detect isolates. Castaneda (1947) also recognized this same problem and developed a two-phase, agar slant and broth, blood culture system to overcome this problem. Although Castaneda designed this system primarily for *Brucella* diagnosis, this system, today termed the «Castaneda Bottle», is used in many laboratories throughout the world for isolation of *Salmonella* as well as *Brucella* and other bacteria from blood.

The Castaneda Bottle blood culture system was recommended by World Health Organization in a monograph by Alton and Jones (1967). Trypticase Soy Broth is one of the media suggested for use in the Castaneda Bottle. Factors inherant in this complete system might modify results somewhat from those found in Trypticase Soy Broth by Kaye et al. (1966).

Morello and Ellner (1969) recommended a new broth medium for blood culture termed Columbia Broth. The ingredients of Columbia Broth were designed to initiate growth from smaller inocula of bacteria and to reduce bacterial generation time, as compared with Trypticase Soy Broth. In addition, they employed the anticoagulant sodium polyanethol sulfonate that was reported to be less toxic to bacteria than sodium citrate. Unfortunately, their test series included only a few specimens from cases of salmonellosis and none from brucellosis patients. As a result, their data for these important pathogens were inconclusive.

The purposes of the present study were to (a) compare the standard Castaneda Bottle culture technique with bile for *Salmonella* blood culture and (b) to compare Columbia Broth with Trypticase Soy Broth for *Salmonella* isolation in Castaneda Bottle systems.

MATERIALS AND METHODS

Culture Isolation Media and Systems

Tryptose Agar/w Thiamine (Difco)* and Columbia Agar Base (BBL)** were prepared, and following autoclaving, slants

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* Difco Laboratories

** BBL Division of BioQuest

of these media were made along one side of 4 oz prescription bottles or milk dilution bottles. Trypticase Soy Broth (BBL) was prepared in the standard manner plus the addition of 5 g of sodium citrate and 50 mg of para-aminobenzoic acid per liter prior to autoclaving. When the broth was cool, 1 ml. of Penicillinase Concentrate (BBL) per liter was added. Each bottle containing a Tryptose Agar slant received 30 ml of broth, added aseptically. Columbia Broth was prepared, and 1 ml of sodium polyanethol sulphonate (Grobax)*** per liter was added. Thirty ml of autoclaved broth was added to each bottle containing a Columbia Agar Base slant. Both types of Castaneda Bottle systems were fitted with vaccine-type rubber stoppers. The air was evacuated from each bottle and a filtered 5-10% CO2-air mixture was introduced. Bottles were incubated for 48-72 hours as a sterility test. The completed bottle is shown in Figure 1. Ox Gall (Difco) was prepared per Kaye et al. (1966). An ox bile culture is demonstrated in Figure 2. Ratios of inocula to culture media were the same as those used by Kaye et al. (1966). However, operational requirements dictated 3 ml blood samples rather than 5 ml. Therefore, 3 ml of blood were placed in 9 ml of 10% ox bile and 3 ml in 30 ml of the broth in Castaneda Bottles.

Cultures were incubated at 37 C. Ox bile cultures were subcultured after 20-24 hrs to Salmonella-Shigella Agar (Difco) and Bismuth Sulfite Agar (BBL) and, if negative, subcultured again after 4-6 days. Castaneda Bottles were observed for growth on the slant at daily intervals and shaken at each observation. When growth was observed on the slant, the bottle was shaken, and a syringe was used to aseptically withdraw a sample of the broth. This was planted on Salmonella-Shigella Agar, MacConkey Agar, and Trypticase Soy Agar plates, shown in Figure 3. The first two media were incubated in air and the latter in 5% CO2. After 21 days of incubation a final subculture was made from each bottle before discard as negative. Isolates were identified by standard biochemical techniques, and their identity was confirmed by agglutination tests.

*** Roche Diagnostics

Fig. 1: Castaneda Bottle

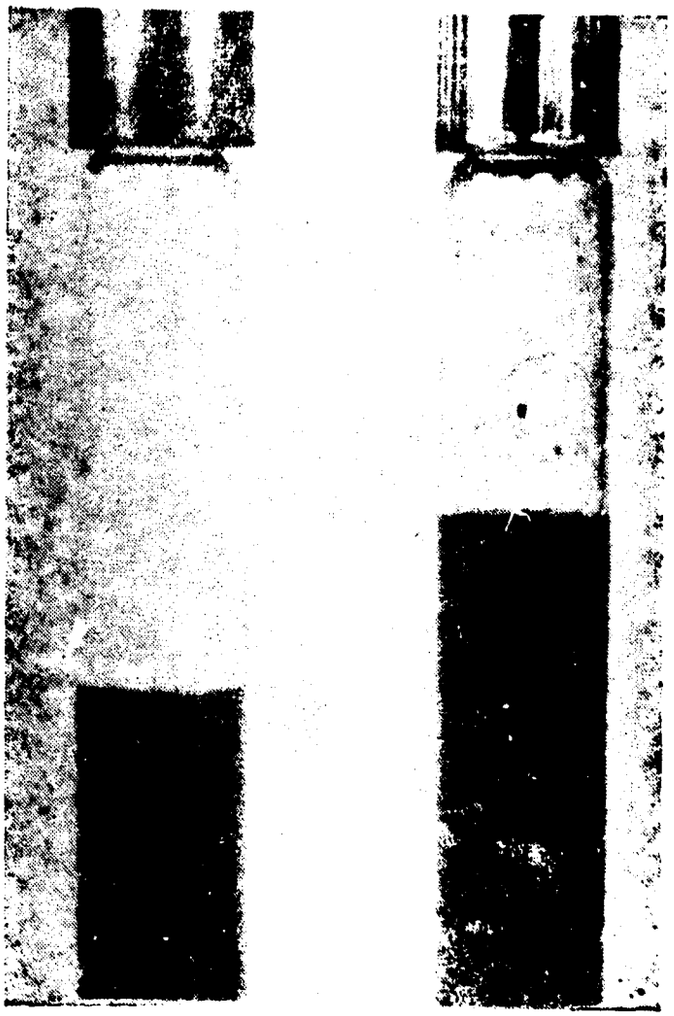

Figure 2: Ox Bile Culture

Patient specimens

Approximately 90% of the positive blood specimens were obtained from clinically diagnosed acute enteric fever patients prior to initiation of therapy. The remaining specimens were obtained from chronic bacteremic cases of typhoid or paratyphoid fever and also chloramphenicol or ampicillin treatment failure patients from the first group.

A few pour-plate cultures were made with 1 ml of blood in Columbia Agar Base (BBL). The levels of *Salmonella* bacteremia observed were about 1-3 per ml, similar to those found by Kaye et al. (1966).

Figure 3: Agar Plates for Isolation of Salmonellae

RESULTS

The comparison of blood culture methods for isolation of *Salmonella* included 701 positive blood specimens. In addition, there were 38 specimens that yielded *Brucella*. The analysis of *S. typhi* cultures presented in Table I demonstrated that the

TABLE I — Comparisin of the Castaneda Bottle with Ox Bile for Isolation of Bacteria from Blood

Etiologic Agents	Specimens Positive	Percent Positive	
		TA/TSB*	OX BILE**
Salmonella typhi	459	84%	72%
S. paratyphi A	238	84%	83%
Salmonella Gr. B	4	100%	0%
Subtotal: Salmonella	701	84%	75%
Brucella	38	100%	0%
Tital	739	85%	71%

* Castaneda Bottle: Tryptose Agar/Trypticase Soy Broth.

** Ox Bil, 10%.

Castaneda Bottle with Trypticase Soy Broth and Tryptose Agar yielded 84% positives while ox bile culture yielded only 72%. This difference was significant ($p = 0.05$). The picture with *S. paratyphi* A isolation was somewhat different, recovery being about the same by each method. Four cultures of *Salmonella* sp. Group B were also isolated, all from Castaneda Bottle culture. Overall *Salmonella* isolations were 84% by the Castaneda Bottle and 75% by ox bile (p between 0.05 and 0.1). When *Brucella* cultures were included, the total isolation figures were 85% and 71% respectively by the two methods.

Some Castaneda Bottles became contaminated, a problem not encountered with ox bile. Contaminants were presumably

normal skin bacteria. Of these 40 contaminated bottles shown in Table II, 17 parallel ox bile cultures were positive.

TABLE II

Analysis of Castaneda Bottle Contamination

Castaneda Bottle Contaminated	Ox Bile Culture	
	Positive	Negative
40	23	17

It was considered that time of blood specimen collection might play a role in the numbers of positive specimens detected. Blood specimens were collected in the morning and about noon from most patients.

There was some difference in isolation rates at these two times from *S. typhi* and *Brucella* patients as shown in Table III. There seemed to be little difference in isolations from *S. paratyphi* A infections.

TABLE III — Bacteremia Related to Time of Blood Specimen Collection

Etiologic Agent	Positive Patient Days	Positive Cultures	
		AM (Fever Peak)	PM (Fever Lysis)
Salmonella typhi	202	78%	89%
S. paratyphi A	67	85%	93%
Sub-Total Salmonella	269	79%	86%
Brucella sp.	15	73%	93%
Totals	284	79%	87%

A series of three to four blood cultures was obtained from 199 patients. The analysis of positive cultures in these series, depicted in Table IV, revealed that the first specimen detected about 85% of the eventually positive patients, while the second

TABLE IV
Positive Blood Culture Diagnoses
Through Multiple Blood Cultures

Blood Specimen	Cumulative Positives
1st	85%
2nd	95%
3rd	98%
4th	100%

specimen increased this to 95%. Further cultures were productive of only a few more positive patients. These cultures included both morning and noon specimens.

The affect of patient therapy on positive blood cultures was studied on 46 positive specimens from treatment failure and relapse patients. The Castaneda Bottle and ox bile methods each yielded 91% positive cultures from these specimens. However, there was a difference in the average days required to demonstrate a positive by the Castaneda Bottle. As may be seen in Table V it required an average of 2.9 days to observe growth from specimens taken prior to initiation of treatment, but from specimens obtained at least 48 hours following completion of treatment, positive visible bacterial growth required an average of 4.3 days. These same periods for ox bile culture were two days each, one day of incubation required for the culture and a second day for the subculture to demonstrate a positive. None of there ox bile cultures required a second subculture to yield a positive test.

TABLE V

Affect of Therapy on Isolation of Salmonella from Blood
(46 Positive Specimens)

Therapy	Days to Positive Culture	
	Castaneda Bottle	Ox Bile 10%
Before	2.9	2*
After	4.3	2

* 1 day incubation for culture + 1 day for sub-culture.

In a series of 35 parallel cultures from positive blood specimens, a comparison was made of the Tryptose Agar/Trypticase Soy Broth media combination versus the Columbia Agar Base/Columbia Broth media combination in Castaneda Bottle blood culture systems. The results indicated in Table VI reve-

TABLE VI

Comparison of Different Culture Media in
Castaneda Bottle Blood Culture Systems
(35 Salmonella Cultures)

Culture System	Positive	
	Percent	Average days to
A: TA/TSB*	91%	2.9
B: CAB/CB**	89%	2.1
A+B	94%	—
A+B+Ox Bile	100%	—

* Tryptose Agar/Trypticase Soy Broth

** Columbia Agar Base/Columbia Broth

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aled that the isolation rate was about the same for each. However, on the average, the Columbia media combination yielded positive results about one day earlier. Neither system yielded 100%. Positive ox bile cultures added another 6% to the combined to total of 94% detected by the two Castaneda Bottle systems.

Seven factors were considered in comparing general applicability of the Castaneda Bottle with that of ox bile for blood culture. As may be seen in Table VII, four factors seemed to

TABLE VII — Cimparison of Operational and Logistic Factors of the Castaneda Bottle and Ox Bile for Blood Culture

Factor	Castaneda Bottle (TA/TSB)		Or Bile 10%	
	Score	Comment	Score	Comment
Growth Efficiency	++	Excellent	+	Good
Culture Observation	+	Direct	0	Sub-culture
Clinical Use	+	Easy	+	Easy
Field Use	0	Difficult	+	Easy
Species Isolated	+	General	0	Salmonella
Preparation	0	Difficult	+	Easy
Cost	0	Greater	+	Lesser

favor the Castaneda Bottle while five favored ox bile. These factors were in relation to our laboratory situation. They might be modified somewhat in another setting.

DISCUSSION

The Castaneda Bottle system as used in this study was significantly superior to ox bile for isolation efficiency of *S. typhi* from blood. With respect to *S. paratyphi* A there was no

difference between the two. Of the 40 contaminated Castaneda Bottles included with negative results in the analysis, 17 had parallel ox bile cultures positive. Since the Castaneda Bottle was more efficient, most of these might have also been positive were it not for the contaminant in each. Since Trypticase Soy Broth was employed by both ourselves and Kaye et al., results of the two studies seemed contradictory. There might have been one or more reasons for this. The media were not strictly comparable, since ours contained para-aminobenzoic acid and penicillinase. The Castaneda Bottle did not require repeated subcultures to detect positives, as did the broth blood culture. Our contamination rate was 5% while theirs was 11%. Kaye et al. noted the problem of introduction of contamination during subcultures from the broth. The additional contamination of Trypticase Soy Broth cultures reported by them may have been in part due to this factor. Had they used a Castaneda Bottle system that would not require subculture to detect positives, more of their contaminated cultures may have been positives, yielding a better result for Trypticase Soy Broth.

However, this still would not account for all the difference. It is well known that a 5% CO2 atmosphere enhances the growth in culture of many bacteria. The increased number of positives in our Castaneda Bottle cultures using Trypticase Soy Broth might, also in part, have been due to the 5% CO2 atmosphere in these bottles.

Another factor might have been related to addition of penicillinase and para-aminobenzoic acid to our cultures, mentioned above. These were not used by Kaye et al. If some of their patients had been on self-treatment prior to the blood sample this might explain their lower rate of isolations in Trypticase Soy Broth as compared with ox bile. Ox bile exhibits neutralizing action on certain antimicrobials and other inhibitory substances in blood specimens.

In the present study, no single culture technique or procedure yielded results approaching 100% detection of positive patients. A series of four specimens was required to give us

all the positive patients included in the study. To be sure, the rate of increase of new patient diagnoses diminished rapidly with succeeding specimen collection efforts, but the conclusion was inescapable that the more specimens cultured, the more positive patients were discovered. Furthermore, when various methods of culture were employed in parallel, more isolations of *Salmonella* were achieved. The best combination to yield maximum results with a reasonable amount of effort seemed to be two serial specimens, morning and noon, on the admission day, these being cultured by both the Castaneda Bottle and ox bile methods. This should detect 95% of theoretically culturally positive patients. Furthermore, it appeared that better than 90% of these positive cultures would be detected after only two days of incubation if the Castaneda Bottle contained the Columbia-type media, at least the Columbia Broth.

The results shown in Table V indicated that prior therapy with chloramphenicol or ampicillin had an affect on bacterial culture, the average days to isolations in the Castaneda Bottle being 2.9 and 4.3 respectively before and following treatment. While these patients had not received therapy for at least 48 hours prior to the specimen, either there was still antibiotic in the specimen, or the surviving bacteria had been altered. This phenomenon occurred in spite of specimen dilution in the broth of the Castaneda Bottle or the penicilliinase present. On the other hand, the factor or factors involved apparently were neutralized by ox bile. These patients will be analyzed in more detail in an attempt to determine more precisely the factors involved.

The relative importance of logistic factors of the two types of culture systems was considered. While the Castaneda Bottle was more efficient in isolating *Salmonella*, it was also more expensive and more difficult to prepare than the ox bile. It was also more subject to contamination, as correctly noted by Kaye et al. Clinical use in established medical practice was equal in ease, but if field work were anticipated, the ox bile would clearly be a better choice. It would be difficult to transport the relatively heavy and bulky bottles, while dry ox bile and test tubes

could be easily transported. Furthermore, requirement for autoclaving Castaneda Bottle components would make the non-autoclaved ox bile a better choice in the field situation, sacrificing some recovery in favor of convenience. On the other hand, in the laboratory it required less technician time to observe Castaneda Bottles for growth than to subculture ox bile tubes. Furthermore, if bacterial species other than *Salmonella* were suspected or their culture were required, ox bile would simply not be suitable. Therefore, blood culture methods chosen must be tailored to best fit the requirements of each situation.

SUMMARY

The Castaneda Bottle and ox bile methods of blood culture for *Salmonella* were compared. Two types of media systems were evaluated in the Castaneda Bottle. More isolations were obtained by the Castaneda Bottle method, but all techniques together were required to yield 100% positive results. Factors related to specimen collection time and numbers of serial specimens were examined, and it was determined that two specimens, one obtained in the morning and the other about three or four hours later on the admission day, those cultured by both Castaneda Bottle and ox bile methods, would yield about 95% of patient culture diagnoses. Logistic comparisons were made between the two techniques.

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EVALUATION OF CHLORAMPHENICOL GIVEN ORALLY AND AMPICILLIN GIVEN EITHER INTRAMUSCULARLY OR ORALLY IN THE TREATMENT OF *SALMONELLA* ENTERIC FEVER

By

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The oral chloramphenicol ampicillin study in the therapy of *Salmonella* enteric fever performed between 1965 and 1967 at the Abbassia Fever Hospital (Robertson *et al* 1968), showed that oral ampicillin therapy of typhoid fever resulted in a 23% failure rate and took roughly one day longer than oral chloramphenicol for full patient response.

It was concluded that chloramphenicol remains the most effective drug in oral treatment of typhoid fever but that ampicillin is an effective alternate drug.

Because of the known dangers of chloramphenicol therapy and because preliminary reports suggested ampicillin to be as effective as chloramphenicol when given intramuscularly

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instead of orally (Kay *et al* 1966), thus overcoming possible intestinal malabsorption of the drug in typhoid fever, it was decided to compare intramuscularly administered ampicillin with orally administered ampicillin and orally administered chloramphenicol thus forming 3 different experimental groups. The study was conducted at the Abbassia Fever Hospital with all laboratory work being performed at NAMRU-3.

MATERIALS AND METHODS

Patients:

Patients were chosen among those presenting themselves at the outpatient department of the Abbassia Fever Hospital, with symptoms of enteric fever. Male and female patients over the age of 4 years were included. The study was conducted during the 17-month period from June 1968 till October 1969, A timespan which includes 2 typhoid seasons as shown in the statistics of Dr. El-Akkad, at this symposium. Patients were placed into one of the 3 therapeutic groups on a strictly non-selective rotational basis as they arrived on the ward.

BACTERIOLOGIC LABORATORY INVESTIGATIONS

Prior to commencement of therapy blood, urine, and stool specimens were obtained for bacteriological examination and handled as described by Mr. Sanborn, at this symposium. After response to therapy and at least 3 days after the termination of antibiotic therapy follow-up blood, stool, and urine cultures were obtained on three consecutive days at weekly intervals. At least two sets of negative cultures were obtained prior to discharge. Mean follow-up time after termination of therapy was in excess of 20 days.

OTHER LABORATORY INVESTIGATIONS

At the time of admission and at weekly intervals thereafter, samples were obtained for complete blood count, Widal

antibody titers, urinalysis, stools for blood, ova, and parasites, and when evidence of schistosomiasis was found quantitative schistosoma egg counts were performed. Male patients were also examined for Glucose-6-Phosphate-Dehydrogenase Deficiency (G6 PD). Other examinations were performed when deemed clinically appropriate.

Therapy:

The 3 drug regimens were administered as follows:

(1) Chloramphenicol 50 mgm per kgm in four divided dosages given orally every 6 hours.

(2) Ampicillin 100 mgm per kgm in four divided dosages given orally every 6 hours.

(3) Ampicillin 100 mgm per kg in four divided dosages given intramuscularly during the first 3 days of therapy then given orally thereafter.

RESULTS

Clinical and laboratory:

Of 449 patients admitted to the study wards, during the study period, 250 had a confirmed diagnosis of *Salmonella* enteric fever made on the basis of positive blood, stool and urine cultures and significant antibody titers. 12 had positive cultures for other organisms than *Salmonella*. Of these 12, 10 had positive blood cultures for *Brucella* and 2 had positive stool cultures for *Shigella*. (Table 1).

Of the remaining 187 patients, many had positive stool cultures for *Salmonella* but had Widal titers that were not considered significant. Others had significant Widal titers but failed to grow out *Salmonella*. Of the non-specific enteric fever group, a large proportion must have had *Salmonella* enteric fever but were not included in the study because the diagnosis was not fool-proof.

TABLE 1

Total number patient admitted	449
Salmonella enteric fever	250
Other fevers	12
Non-specific enteric fever	187

Of the 250 patients with confirmed diagnosis, 227 or 91% were diagnosed on the basis of one or more positive blood cultures, 23 or 9% were diagnosed on the basis of positive urine and/or stool cultures for *Salmonella* plus a significant Widal titer, which meant a titer to any of the antigens used of at least 1 to 640. (Table 2).

TABLE 2 — 250 Patients with salmonella enteric fever diagnosed on the basis of:

Positive blood culture	227	91%
Positive urine or stool culture plus significant widal titer	23	9%

The causative organism in the 250 cases were *S.Typhi* in 193 or 77% of the cases, *S.Para A* in 50 or 20% of the cases, *S.Para B* in 4 or 1.6% of the cases. In our series we found that a significant number of culture proven *S.Para A* patients had equal or higher Widal antibody titers to *S.Para B* than to S Para A. Had we based the diagnosis *of S.Para B* fever on Widal titers and not on cultures, we would have had a much higher proportion of *S.Para B* patients. The remaining 3 patients grew out *S.Montevideo*. (Table 3).

TABLE 3 — Salmonella isolated in 21 patients with salmonella enteric fever

Salmonella typhi :	193	77%
Salmonella Paratyphi A :	50	20%
Salmonella paratyphi B :	4	1.6%
Salmonella Montevideo :	3	1.3%

The patient profile in the 3 therapy groups were as follows: There were 84 patients in the chloramphenicol group, 90 in the oral ampicillin group, and 76 in the intramuscular ampicillin group.

Female to male ratio was 1 to 1.5 or 2 females to 3 males. in other words a predominance of males as found in the nation wide statistics of the U.A.R. as pointed out by Dr. El-Akkad.

Mean age was comparable in the 3 groups. The mean age of 12.5 years places our study group in the older pediatric age group. There was one patient of 3 years who was a sibling of an older patient included in the group, otherwise, all patients were over 4 years of age as mentioned in the design of the study. There were few patients over 25 years of age in spite of the fact that no effort was made to select younger patients.

Duration of fever before treatment:

Patients had been ill with fever for an average of one week prior to admission. A few, not more than 5% of the entire group gave histories of being ill with fever over 4 weeks prior to admission. (Table 4). A significant number gave previous history of febrile illness: possibly previous attack of enteric fever.

TABLE 4.

		Sex Distribution		Duration of fever Before treatment		Mean age and range	
	No.	Female	Male	Mean	Range	Mean	Range
Chloramphenicol	84	1 :	1.5	7.5 Days	2 — 30	12.5	4 — 35
Ampicillin oral	90	1 :	1.4	7.7 Days	3 — 30	12.5	3 — 50
Ampicillin IM	76	1 :	1.7	8.1 Days	3 — 30	12.3	4 — 24

THERAPEUTIC

Response to therapy:

Response to therapy was counted as the first of 3 consecutive days when the oral temperature remained below 37.6 degrees (centigrades). Therapy was continued for 7 days after defervescence. Treatment failures were considered in patients where no response was seen within 10 days of commencement of therapy. The fact that time before defervescence ranged up to 14 days was because in those patients a definite response was seen prior to the 10th day of therapy, but that during the 7 days of therapy after initial defervescence, a minor temporary temperature elevation was seen, therefore, postponing the day of defervescence to after the normal 10 days that would have been counted as a drug failure. (Table 5).

TABLE 5 — Duration of therapy before first afebrile day

	Mean	Range
Chloramphenicol	5.4 Days	3 — 11
Ampicillin oral	7.9 Days	2 — 14
Ampicillin IM	7.2 Days	2 — 14

The time required for response to therapy was 2 days longer in either of the ampicillin groups compared to the chloramphenicol group. This rather significant difference was already evident after cursory examination of the fever charts of the study patients. Because one of the main purposes of this study was to compare orally administered ampicillin with intramuscularly administered ampicillin, it is important to note that no significant differences existed in the response to the two drug regimens.

In spite of ampicillins proven *in vitro* activity against *Salmonella,* it is still less effective than chloramphenicol.

Drug failures:

Drug failures as defined earlier included patients not responding to therapy within 10 days after commencement of treatment. There were 1 or 1.2% in the chloramphenicol group. There were 7 or 8% in the oral ampicillin group and 9 or 12% in the intramuscular ampicillin group. Sex ratio and causative organisms are shown in table 6.

TABLE 6 -- Drug failures in 250 patients with salmonella enteric fever

Drug	Total	Drug Failures		Sex	Organism
		No.	%	F : M	
Chloramphenicol	84	1	1.2%	1 : 0	1 S. Typhi
Ampicillin oral	90	7	8 %	2 : 5	6 S. Typhi
					1 S. Para A
Ampicillin IM	76	9	12 %	4 : 5	8 S Typhi
					1 S. Para A

Drug reactions:

There were no drug reaction in the chloramphenicol group. In the previous study at Abbassia Fever Hospital and NAMRU-3, 3 patients with severe hemolytic anemia were found in chloramphenicol treated patients. No evidence of significant hemolysis was found in our patients.

13 or 14% of the oral ampicillin group and 4 or 5% of the intramuscular ampicillin group had mild reactions to ampicillin consisting mostly of benign drug rashes responding to treatment without necessitating the discontinuance of ampicillin. (Table 7).

TABLE 7.

	Total No.	Drug Reactions	
		No.	%
Chloramphenicol	84	0	0
Ampicillin oral	90	13	14%
Ampicillin IM	76	4	5%

Relapses defined as symptoms, fever, and bacteremia after the cessation of therapy occurred in an equal proportion of patients in each drug group as shown in table 8.

TABLE 8 — 35 Relapses divided according to drug regimen

	Total No.			F : M
Chloramphenicol	84	12	14%	3 : 9
Ampicillin oral	90	13	14%	4 : 9
Ampicillin IM	76	10	13%	1 : 9

The relationship between schistosomiasis and recurrent *Salmonella Para A* fever has been shown by Dr. Saad Hathout *et al* 1967), and has again been discussed by Dr. S. Hathout, Dr. Z. Farid, Dr. J. Lehman, Jr., and Dr. S. Botros at this symposium. In this present series this relationship was again shown by the relative predominance of patients with *Salmonella Para A* fever and schistosomiasis among the relapse. (Table 9).

TABLE 9 -- 35 Relapses divided according to organism and Presence of Schistosomiasis

	Total		Schisto	
		F : M		F : M
S. Typhi	20	(7 : 13)	4	(2 : 2)
S. Para A	15	(1 : 14)	12	(1 : 11)

There were 4 deaths in the series. They all occurred in the *Salmonella* enteric fever group. None occurred in the non-specific enteric fever group.

One death occurred in the chloramphenicol treated group. This patient was a 13-year old male patient who had been ill 6 days prior to admission. He died on the 14th hospital day. Autopsy findings showed bowel changes consistant with *Salmonella* enteritis. There was an abscess of the left lung. Culture of a swab of the bowel taken at the postmortem examination grew *Salmonella Montevideo.*

Two patients died in the oral ampicillin group. One was a five years old female, ill for 10 days prior to admission who died on the fifth hospital day. Blood cultures grew *Salmonella Typhi* No autopsy was performed. The other patient was a 27-year old male ill for 7 days prior to admission who developed gastrointestinal bleeding and intestinal perforation on the fourth hospital day. He was subsequently treated with

chloramphenicol. He died on the sixteenth hospital day. Initial blood and stool cultures grew *S.Para A.* Autopsy showed typhoid ulcers of the intestine and intestinal perforation.

One patient died in the intramuscular ampicillin group. She was a 20-year old female who had been ill for 5 days prior to admission. She died on the fourth hospital day. Initial blood cultures grew *S. Typhi.* Autopsy showed *Salmonella* enteritis.

10 patients in the chloramphenicol group, 3 patients in the oral ampicillin group and 7 patients in the intramuscular ampicillin group were immediate post-therapy or transcient carriers having positive stool or urine cultures up till 2 months after cessation of therapy. A majority of these patients were 1 or 2 week carriers that subsequently had two sets of weekly negative stool and urine cultures without further therapy.

Male patients had G6PD screening test performed on the day of admission. All positive screening tests (Cyanide ascorbate test as described by Jacob and Jandl) had G6PD assay performed during convalescence (UV absorption (TNPH) test at 340 millimicrons).

Of 94 patients tested 25 had positive screening tests and of these 25, 6 patients were G6PD deficient by assay. The number of patients tested is not large and there is no significant difference in G6PD deficiency compared to another series performed here in Egypt on normal patients.

CONCLUSIONS

There does not seem to be any difference in the efficacy of oral and intramuscular ampicillin in the treatment of *Salmonella* enteric fever. Drug failure rate is higher and the time required for response to treatment is 2 days longer when either oral or intramuscular ampicillin is used as compared to oral chloramphenicol. Probably intestinal malabsorption does not play a

role in the poorer response to oral ampicillin. Serial blood samples were obtained during this study on the 1st and 2nd day of antibiotic treatment for serum ampicillin and chloramphenicol levels. These samples are still in the process of being assayed. No cases of severe hemolysis were seen in our chloramphenicol treated patients as compared to the group seen by Robertson *et al.*

Although mild in nature the rate of drug reactions is higher in the ampicidlin groups. There were no significant differences in the rate of relapse or carrier status in the 3 drug groups. However, the relapses and carriers found in this study need to be more thoroughly studied with regards to schistosomiasis and *Salmonella Para A* fever, and will be the subject of a separate report. A cursory look at the data suggests that in a group of patients with *Salmonella* enteric fever like this one there is a significant number of patients with schistosomiasis and chronic salmonellosis due to *S. Para A* and that this patient group plays a significant role in increasing the relapse rate and possibly also the chronic carrier status.

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ANTIMICROBIAL THERAPY OF SALMONELLA INFECTIONS

By

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Excellency, The Minister, Mr. Chairman, Ladies and Gentlemen:

The program committee has been generous in assigning me this topic because I am not restricted to any type of clinical syndrome caused by Salmonella and can comment on all aspects of the therapy of these infections. Although I will emphasize recent developments in therapy, many of my comments will be concerned with unsolved problems related to older therapeutic measures, some of which have been in use for more than twenty years.

First — a few comments about chloramphenicol. This drug, used in the treatment of typhoid fever since 1948 when the original observations were made by Woodward and colleagues (1, 2, 3), has occupied a position of first choice in the therapy of systemic Salmonella infections for over 22 years. To my knowledge, this position of leadership has never been seriously or lastingly challenged by any other antimicrobial, either because

of the development of a significant proportion of chloramphenicol-resistant strains or because of the development of a more effective, more quickly acting or less toxic drug. The most meaningful challenge to chloramphenicol to date has come from ampicillin, and this will be discussed in subsequent paragraphs.

It should be noted that despite the prolonged period of study of chloramphenicol in the therapy of typhoid, we still do not have all of the answers regarding dosage and duration of therapy. Had I been asked the optimal dose of chloramphenicol and the optimal duration of therapy in typhoid fever, I would have answered, «50 mg per kg for two weeks without a loading dose». Yet at this conference, remarkable disagreement on these points has been expressed from individuals with long experience in the treatment of this disease. Although I would want to hold to the view that «50 mg per kg for two weeks» is correct, the differing opinions that I have heard cause concern, lead to re-evaluation and make me seek explanations for the differences of opinion. Some of the apparent differences in recommendations regarding duration of therapy seem to be related to differences in the manner of expression of «duration of therapy». In my opinion, it is confusing to define duration of therapy, as is so often done throughout the world, in terms of the number of days after the patient becomes afebrile (e.g., «therapy should be continued for five days after the patient becomes afebrile»). There is tremendous variability, ranging from one to more than seven days, in the duration of fever after beginning chloramphenicol in patients with typhoid fever. Obviously duration of therapy based on duration of fever is also variable and results in a series of this type cannot be compared with results in a series of patients treated for a definite, constant period of time. Although the fever curve provides an excellent parameter of response, experience with other infections indicates that cure can better be correlated with total duration of antibiotic therapy than with other clinical parameters. Another reason for differences of opinion regarding the dose of chloramphenicol and the duration of therapy relates to the tendency to regard all patients with typhoid fever as being alike and which leads us to expect the same results in therapy from one patient to the next and

from one country to the next. This approach, as has been pointed out many times at this conference, is fraught with difficulties because the rapidity of response and the outcome of therapy is influenced by the duration of illness when therapy is started, the presence of associated diseases (e.g., schistosomiasis), the virulence of the organism, the age of the patient, the duration of therapy, the quality of chloramphenicol used, and by other factors such as the immune status of the host.

Another old and important question is, «What is the influence of chloramphenicol on the incidence of relapse and the chronic carrier state?» This question has been asked and answered many times (3,4,5,6,7,8). There is general agreement that the incidence of the chronic enteric carrier state with a persistent site of infection in the gallbladder is not altered by chloramphenicol, this remaining at about 2 to 3 per cent of the patients with typhoid fever. In contrast, there is considerable variation in opinion regarding the incidence of relapse after chloramphenicol therapy of typhoid fever. The factors that determine relapse are multiple and inadequately studied. A reasonable generalization is that before the advent of chloramphenicol approximately 8 to 10 per cent of patients with typhoid underwent relapse in the absence of any microbial therapy, whereas 9 to 20 per cent of patients receiving chloramphenicol undergo relapse.

Thus, although chloramphenicol is our best drug in typhoid, it is not ideal because of the occurrence of chronic carriers, which are a tremendous public health menace, the high incidence of relapse in the range of 9 to 20 per cent, the slow response, and the toxicity of the drug. There are many reasons to search for a more effective antimicrobial that would eradicate the typhoid bacillus, rapidly terminate the acute illness, and reduce the incidence of the carrier state and relapse to zero.

In searching for a new drug, it has become obvious that we cannot rely on *in vitro* sensitivity tests as a guide. Although *in vitro* sensitivity tests provide the best guide for the selection of an antibiotic in the treatment of many infections, typhoid fever is one of the best examples of a microbial disease in which *in*

vitro sensitivity and *in vivo* response to an antimicrobial do not coincide. Despite the fact that the typhoid bacillus shows equal sensitivity to two drugs, for example, tetracycline and chloramphenicol *in vitro* (8), the response *in vivo* may be, and is in this instance, decidedly different. As is well known, the response to chloramphenicol is relatively predictable, in that most patients become afebrile three to five days after beginning chloramphenicol; in contrast, patients treated with tetracycline show either no response or a very slow response. Thus, it is well accepted that *in vitro* sensitivity of Salmonella serotypes does not necessarily correspond to *in vivo* response. The other aspect of this question, that is, whether *in vitro* resistance is always associated with *in vivo* resistance has also not been completely answered, although the evidence available at present indicates that there is little likelihood of *in vivo* response if the infecting organism is resistant *in vitro*.

A number of drugs that have shown good or reasonable *in vitro* activity against *Salmonella typhosa* and either no effect or poor results in patients with typhoid fever are, in addition to the tetracyclines (5), cephaloradine (10,11,12,13,14,15), streptomycin (5), kanamycin, the polymyxins (5, 10), gentimicin (10), nalidixic acid (10), paromomycin (10), the sulfonamides (5), and penicillin G, usually in small doses (5). One other drug, furazolidone (Furoxone) (16,17,18,19,20), can probably be added to this list although it has been studied more extensively and has shown more promise than most of the other drugs. Several series, notably those of Chakrabhorty (18), Budzhe and associates (19), and Drs. Omar and Wahab (20), the latter study carried out at the Abbassia Fever Hospital, have shown that furazolidone exerts a beneficial effect on the course of typhoid fever. However, the response as judged by the duration of fever is less rapid than with chloramphenicol. In addition, these reports indicate that 5 to 10 per cent of the patients fail to respond to therapy, that new problems of toxicity are introduced, and that relapse continues to occur. When all of the evidence is considered, it appears that furazolidone occupies at best a tertiary position in the therapy of typhoid fever.

The most serious threat to the position of chloramphenicol as a therapeutic agent in typhoid came early in the 1960's with the development of ampicillin, a semi-synthetic penicillin, with striking *in vitro* activity against *Salmonella typhosa* and other Salmonella serotypes. Considerable experience is now available with ampicillin, this beginning with the studies of Scioli and colleagues (21), Sleet and associates (22), Patel (23), Sanders (24), Kaye and colleagues (25), and extending to those of Robertson, Wahab and Raasch (26,27), and Sorensen and colleagues at NAMRU (presented at this conference), both of the latter excellently controlled studies comparing ampicillin with chloramphenicol. It seems established that many patients with typhoid fever do indeed respond to oral ampicillin but the response is slower than with oral chloramphenicol. In the studies of Robertson and colleagues, the febrile period lasted an average of 5 days in patients treated with chloramphenicol and 6.5 to 8 days in patients treated with ampicillin. In addition to the slow response to ampicillin, some patients do not respond at all to this drug during the initial phases of therapy. This apparent lack of response seems to occur in 5 to 10 per cent of patients treated with oral ampicillin. Although the same phenomenon occurs in occasional patients treated with chloramphenicol, it is much less common — perhaps occurring in only about 1 per cent of the patients. So, based on this and other evidence, ampicillin appears to occupy a second choice position among antimicrobials used in the therapy of typhoid fever and constitutes a suitable alternative drug for patients with typhoid who cannot take chloramphenicol. Additional information is needed regarding the influence of ampicillin on the relapse rate and the chronic carrier state. It was initially hoped that this bactericidal drug might actually reduce the incidence of relapse and the chronic carrier state, but despite initially promising results, the present evidence suggests that ampicillin is no better than chloramphenicol in this regard.

Because of the slow response to chloramphenicol, the high relapse rate, the occurrence of chronic carriers, the slight risk of severe bone marrow toxicity, and the potential threat that the typhoid bacillus could become resistant, each new antibiotic

should be evaluated as a potential therapeutic agent in typhoid fever and other Salmonella infections. At the present time the combination of trimethoprim and a sulfonamide seems to offer some promise and obviously requires careful clinical evaluation. Trimethoprim is one of a group of compounds which were first fully described in 1962 by Roth and colleagues (28). Drugs in this group have both antimalarial and antibacterial activity; perimethamine is noted for high antimalarial activity whereas trimethoprim shows greater antibacterial activity. Trimethoprim alone is bacteriostatic, as are the sulfonamides, but when combined with sulfonamides, is said to be bactericidal in low concentrations (0.2 micrograms per ml) (29,30,31). Both compounds act at separate stages in the biosynthetic pathway of folic acid. which is necessary for DNA synthesis. The sulfonamides inhibit the conversion of para-aminobenzoic acid to dihydrofolic acid and trimethoprim blocks the conversion of dihydrofolic to tetrafolic acid. Clinical information regarding the efficacy of the combination of trimethoprim and sulfonamides in the treatment of bacterial infections is scant at present but promising results have been obtained in patients with urinary tract infections, bronchitis, and certain other soft tissue infections (31). Several preliminary reports have appeared on the use of this combination in the therapy of typhoid fever (32,33,34). Although these are not controlled studies, the patients treated with the combination apparently did just as well as patients treated with chloramphenicol, and, in fact, fever seemed to subside faster in the patients treated with the combination than in patients receiving chloramphenicol alone. Although there is every indication for a cautious attitude in evaluating preliminary results of antimicrobial trials in typhoid fever, the combination of trimethoprim and sulfonamides appears to be promising and careful evaluation of these compounds in the therapy of typhoid fever is required.

I would now like to turn to some comments regarding the therapy of chronic Salmonella carriers. The chronic enteric carrier state refers to patients who continue to excrete Salmonella in their stools for periods of one year or more. This phenomenon occurs in approximately 3 per cent of the patients with typhoid fever observed in Europe and in the United States. Most chronic

enteric carriers are related to infection with *Salmonella typhosa* although at times *Salmonella paratyphosa A, B,* or *C* may be involved. The chronic enteric carrier state practically never occurs as a consequence of infection with other salmonella serotypes in adults. Once organisms have been demonstrated to be present in the stools for one year or longer, it is likely that continued excretion of organisms will continue for years, often for the life of the individual. We have studied a group of chronic enteric typhoid carriers in New York City in whom the duration of the carrier state ranged from several years to more than forty years (35). The number of organisms excreted in the stools of these individuals is exceedingly high, usually always in the range of one million viable units of bacilli per gram of feces and frequently in the range of one billion viable units per gram. The source of organisms in the stool is the biliary tract, and the number of organisms present in bile obtained from the duodenum after contraction of the gallbladder is exceedingly high.

We have evaluated therapy of the chronic enteric carrier state with ampicillin (8). In this series a group of twenty-four patients were treated for a period of six weeks with ampicillin in a daily dose of 6 grams combined with probenecid. The ampicillin was given in a dose of 1.5 grams four times a day, and the probenecid in a dose of 0.5 grams four times a day. After a period of several days, stool cultures in all patients were found to be negative for typhoid bacilli even when penicillinase was added to the culture. When the stools became negative for *S. typhosa,* negative cultures continued throughout the period of therapy. However, when therapy was discontinued the incidence of relapse was quite high and usually occurred within three months after discontinuation of therapy. These patients have now been followed for periods of three to four years and the results of therapy can be summarized as follows: «Cure» was achieved in 9 of the 24 patients after one course of therapy. Six of the patients who relapsed after a single course were re-treated in a similar manner and of these 3 of 6 were apparently cured. Thus, of the total of 24 patients, 12 were apparently cured of their chronic carrier state with the combination of ampicillin and probenecid administered for a period of six weeks.

The results of therapy were influenced by the presence of gallbladder disease. All of 7 patients who had normal cholecystograms with prompt filling of the gallbladder and no evidence of gallbladder disease were cured with ampicillin. In contrast, only 5 of 17 patients who showed non-visualization of the gallbladder or gallstones were cured. «Cure» of the chronic enteric carrier state is exceedingly difficult to document because relapse may occur at a late date. In most patients relapse occurred within three months after the termination of therapy, but in one patient relapse occurred after one year of observation and in two patients relapse occurred after two years of observation with repeated negative stools during the intervening period.

These results with ampicillin are somewhat similar to those obtained by others (7,36), although the rate of cure varies depending on the presence or absence of gallbladder disease, the duration of the carrier state, the length of therapy, and perhaps many other factors(8). We feel that the position of ampicillin in the therapy of the chronic enteric carrier state is as follows: In patients who show no evidence of gallbladder disease and prompt filling of the galbladder on cholecystogram prolonged ampicillin therapy constitutes the approach of choise and can result in «cure» in most patients. In contrast, the incidence of cure is exceedingly low in patients who show evidence of gallbladder disease; in these patients, cholecystectomy constitutes the therapy of choice. Cholecystectomy alone results in cure of the chronic enteric carrier state in approximately 85 per cent of the individuals even in the absence of antimicrobial therapy although in most series some type of antimicrobial therapy has been administered during and immediately after the period of cholecystectomy.

The therapy of urinary carriers of Salmonella has been discussed by others at this symposium and will not be considered here.

Finally, let me turn to the question of the therapy of Salmonella gastroenteritis, the most common type of Salmonella infection. There is adequate evidence at the present time to sup-

port the view that antimicrobial therapy irrespective of type does not exert a beneficial effect on the course of Salmonella gastroenteritis. Antimicrobial therapy apparently does not result in a decrease in the duration of illness or a decrease in the duration of excretion of organisms in the stool after symptoms have subsided. In fact, increasing evidence would indicate that antimicrobial therapy may exert a deleterious effect on the course of intestinal infections with Salmonella. These studies (37, 38, 39) indicate that the period of excretion of organisms in the stool during convalescence after symptomatic Salmonella infection is actually longer in patients who have been treated with antimicrobial drugs during the acute illness than in patients who have received no antimicrobial therapy. The antimicrobial therapy apparently interferes in some way with the clearing mechanisms which normally operate in the intestinal tract for Salmonella. Another study (40) carried out in Swedish tourists visiting other areas of the world showed that the incidence of Salmonella infection was actually higher in patients who had been treated with oxyquinolines as a prophylactic measure than in those who received no antimicrobial therapy (17 per cent vs. 28 per cent).

In patients with the more serious systemic non-typhoidal Salmonella infections, chloramphenicol continues to be the antimicrobial agent of choice. However, ampicillin does exert a beneficial effect on the course of the disease in most patients and can be used in patients who cannot be treated with chloramphenicol.

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ROUND TABLE DISCUSSION ON PUBLIC HEALTH CONTROL — CRITIQUE FOR FUTURE RESEARCH*

HELD ON 15 JANUARY, 1970.

* Due to limitation of space, only the comments of the Panel Members have been published. Unfortunately, questions and comments from the floor, although interesting and pertinent, had to be omitted.

DR. FARAG RIZK: Gentlemen, the subject of the round table discussion we will have now is «Public Health Control». I think you will all agree with me that the best way of doing it, with the time we have, is for each speaker to present his views on the subject, and then the floor will be open for general discussion.

May I call first on Dr. Hook and then Dr. Hornick, Dr. Nicolle, and Dr. Kamal. We will then be honored by final comments from H.E. The Minister of Public Health. The floor will then be open for discussion. The speakers are all here at the table, so now I call on Dr. Hook.

DR. EDWARD W. HOOK: As far as this problem is concerned I really won't have to talk very long as I don't have any

brilliant ideas to bring up that have not already been discussed. It seems to me that during these three days we have covered most aspects of typhoid fever and have had many new and interesting ideas brought forth. In our present state of knowledge, it is quite clear that the antimicrobial therapy available offers, really, very little in the way of control. Thinking not just in terms of utilization of antimicrobials as prophylactic agents, which I think have been so unsuccessful in many areas, but also we really don't have antimicrobials that alter the chronic carrier state, which is terribly important in the perpetuation of this disease, whether it is in the urinary tract or the biliary tract or elsewhere. I think that Dr. Hornick's remarks and what others have mentioned have painted a rather dismal picture for us as far as vaccines are concerned. The vaccines that we have, really, do not evoke a strong immunity. We can always look forward to the development of new vaccines, but they do not seem to be clearly on the horizon. One aspect of this which does not relate directly to vaccines but which would perhaps provide us with leads which would be helpful in control, relates to understanding the mechanism of immunity in patients with typhoid fever. There is no question but that immunity does exist, but we do not understand the mechanism by which this is brought about. More efforts towards understanding the basic mechanisms of immunity would, in the long run, be very helpful in the control of this disease. It is obvious that with these comments I have made that we are perhaps left with what is, I guess, the best control measures of all. Those are the things which we would group under public health control measures, Thank you.

DR. RICHARD B. HORNICK: I would like to echo what Dr. Hook has said in terms of vaccine effectiveness as a good measure to cut down on the incidence of typhoid fever. I was a little surprised at the statistics of typhoid fever in Cairo. There really did not seem to be any diminution in the incidence of the disease in the last decade. This surprised me somewhat, because the water supply here is obviously excellent. This would suggest that, unlike other areas of the world where typhoid is

mainly water-borne, perhaps in Cairo water-borne typhoid is least likely. This is perhaps not so in the rural areas.

This leads to the question of what we can do about carriers. Here again the problem of preventing the carrier state was dealt with by several discussants at this symposium. We are still left with the problem as to how to prevent the development of the carrier state. In our country it is more likely to be the women who become carriers because of their greater chance of having biliary tract disease, and the older they are, the more children they've had, the more difficulties with the biliary tract, the more likely they will be to be carriers. Certainly behind every typhoid case there has to be a human source, there has to be a carrier somewhere. It seems very simple to state the obvious fact that if we can clear up all the carriers, then typhoid fever should disappear. I would think that the one public health control measure that we need here, in addition to the water supply which has already been controlled, is to try to do studies to first identify the carriers and control their type of occupation so that they will not be spreading the disease through food handling. A concentrated effort should be made to apply our current therapeutic measures to try to cure these carriers, to try to diminish the foci of infection. I think this is just like other infections. When the focus of infection is eliminated, then the disease itself is obviously not spread. So, I think the focus here must be the carriers, and we have to devise some way of finding and identifying them, and perhaps either curing them or at least control their occupations, so they will not spread the disease. Thank you.

DR. FARAG RIZK: Thank you. Dr. Hornick. Professor Nicolle, you have the floor.

DR. PIERRE NICOLLE: It is phage typing *of Salmonella* that concerns me. One of the first problems is to obtain the set of Vi phages from the International Reference Laboratory. In this case there is a psychological problem, political too, maybe. Yet, these obstacles are not necessarily insurmountable. It is possible to solve them. In case you do want a phage typing center in Cairo. I suggest that you apply to Dr. Anderson, and

probably you might be able to obtain this. Dr. Anderson is the Head of the International Phage Typing Reference Laboratory in England. I think that you should apply directly. However, if I can intervene I shall do so. My former laboratory, being that I am now retired. is willing and capable to accept any amount, hundreds or thousands of cultures, for phage typing if you should decide to do so. However, that would only be a temporary solution. Certainly, phage typing is very important to epidemiology in combat against *Salmonella* enteric fever. This method is presently being used in 70 centers in the world. I think that the number of phage typing centers indicates that the method is valuable and has its uses. There are several phage typing systems, those for Salmonella *typhi, S. paratyphi A, S. paratyphi B* and *S. typhimurium.*

DR. FARAG RIZK: Thank you very much, Dr. Nicolle. Now I call on Dr. Kamal to give us his views on the subject.

DR. A.M. KAMAL: It is said that history is the best teacher. If we look at the history of typhoid in the already highly developed countries where, for example, in certain areas in the U.S. and in the British Isles, there are very few or no typhoid cases. Most of these few are imported. Why? What did they do?

During the 19th Century, and early in this Century, there has been a big upheaval of industrialization. This brought up environmental sanitation. Public health measures did not begin with vaccines, did not begin with isolation of contacts or isolation of patients, did not begin with treatment. Public health began only with environmental sanitation, cleansing of the environment. England, began, then the U.S. followed England. They also began with improvement of environmental sanitation, with the betterment of environment, and with the social economic status of the people, Then education entered. What I mean by education is public education, ordinary education. You must have knowledge. Now these two elements, the betterment of environment sanitation combined with the rising standard of the socio-cultural level

of the population, were the push, the first impetus if not the main factor in getting rid of enteric infections in general, including typhoid.

When I say environmental sanitation, I do not mean only potable water supply, I do not mean only healthy disposal of refuse, or proper disposal of excreta. I do not mean only housing. I mean all these items. If you bring water to any community, clean potable water, and leave out the other items of environmental sanitation, it won't have any effect. Environmental sanitation is a unit. So, if we want to clean the environment and make it healthy, then we must tackle all the items together. Otherwise, the effect of bettering or improving one item alone will not give us the result which we seek. If we improve the environmental sanitation, if we educate the people, if we raise the socio-economic status of those people, then personal hygiene automatically will rise. This uplift of personal hygiene together with the betterment of environmental sanitation will do away with typhoid. This is not a matter of clearing carriers. They did not do this in America, and they did not do this in England.

If we go by what I am proposing, then the number of new cases will drop. With the fall of new cases, the carriers will drop, and then we will be left only with the chronic carriers. These will die with age. If you have in a community, for example, 50 chronic carriers, in 10 years they will be 20. In 15 years they will be three. Then we will be free of this source of infection. That's what has been done outside, and that's what we ought to do. The matter of vaccination you have heard. Treatment is all right. Treatment is humanitarian. You must treat the cases. However, if we want to control the disease or if we want to eradicate it, there is nothing we can do except the betterment and improvement of all points in environmental sanitation plus education. These are the two main factors that could really bring us to the goal we seek, eradication of not only typhoid but all enteric diseases. Enteric diseases are dirt diseases. If we get rid of dirt, if we get rid of ignorance, then we could get rid of enteric infections as a whole, typhoid included.

DR. FARAG RIZK: Thank you very much, Dr. Kamal. Of course, the principles of public health control are well known, and there is no disagreement about it. However, emphasis and stress in application varies from country to country according to environmental conditions. Dr. Hook stressed the importance of carriers with the understanding that there is a good water supply in most places in this country. I think that this may be true in a condition with a better environmental sanitation standard where typhoid mostly occurs as isolated outbreaks. However, in a condition where typhoid is endemic, where the environment has many defects, where flies are prevalent and insanitary disposal of excreta is present, we have many cases. These are not only the true cases but also the mild, modified, or subclinical cases. These are important sources of infection, as important as carriers in a country like this. When you consider carriers ,the number of carriers in this country is tremendous I am sure. So, I wholly agree with Dr. Kamal that we should put great stress on correction of sanitary defects of our environment here. What we can do about carriers with the present state of knowledge is to prevent them from acting as food handlers. This, we are doing here. It needs more stress, I confess, but this is what we can do with them right now. Listing carriers, as it is done in some metropolitan cities abroad, and trying to treat them is a measure beyond near feasibility. Of course, prevention of carriers from acting as food handlers will definitely help. But here again it will not give us 100% satisfaction, because people do not always eat outside as they do in many countries abroad. People eat inside their homes and eat in places which are not licensed. So, if we only prevent carriers from acting as food handlers in those licensed places, you will have improvement, but you will not have the whole improvement we expect. So, I wholly agree again with Dr. Kamal, we should put more and more stress, as much as we can, on improvement of the environmental conditions. We should prevent carriers from acting as food handlers.

Thirdly, what is the place of vaccination in a disease like this, in our country? Should we just forget it, or should we practice it to a greater or smaller extent? Perhaps we should

vaccinate under certain circumstances. What are these certain circumstances? I confess, I don't have the clear answer to this. Every point of view has its advocates. It is a question that may be tackled by the group after we have been honored by the comments from His Excellency, the Minister.

H.E. THE MINISTER OF HEALTH, DR. ABDOU M. SALLAM: Mr. Chairman, thank you for giving me the chance to say a few words. Being a clinician originally, I feel a bit out of place. I am really flattered and honored to be amongst this group of scientists discussing this subject. I would like to take this opportunity to thank NAMRU-3, very much, for giving this chance to me personnally, to our country, and to our scientists at Abbassia and at Embaba Hospitals. I feel that cooperation in such fields can stir up a lot of interest amongst people working in the same field, permitting them to cooperate together and to follow developments and new ideas from all over the world. I am especially grateful to NAMRU-3 for this opportunity, bringing to us eminent workers like Dr. Hook, Dr. Hornick, and Dr. Nicolle. I feel this is putting this seminar into contact with International developments, and I feel that together, working in the same field, we shall advance and link our ideas and our work with developments abroad.

Now, about the subject itself, of course typhoid is a very important subject for us in our country, and especially for me representing the Ministry of Public Health, trying to defend the people from such diseases. I did not participate in all the talks, and I am waiting until what has been said in all the discussions which have taken place in this seminar will be summarized and published to see how to make best use of all that has been said. As regards control, let me confess that I am still in the dark, and remembering what Dr. El-Akkad said, for instance, in the first talk I attended, showing some statistics about the prevalence of typhoid in the U.A.R. From what he said and from what I heard here after that, I feel strongly the need for finding and carrying out ways and means to make better control.

Now, we are talking about control, not about eradication. A few days ago, we were talking about malaria at the Ministry, discussing whether what we were intending would be control or eradication. However, I feel satisfied talking about control when we are talking about typhoid fever. One feels that there is a lot more that needs to be done to try to reach anything new concerning control. Mr. Chairman, what you said about the role of vaccination and about the role of those who are handling food makes the problem of control more vague in my mind. It also makes measures available and applicable for proper control, effective control, in need of elucidation. It was a bit distressing to see the statistics about the prevalence of typhoid fever in the U.A.R. today, in Cairo and especially in Alexandria. Statistics showed the number of cases between 1964 until now. I wonder how the numbers compare with the prevalence before 1964. My feeling was that cases were even becoming more, not less, and I feel that we are getting less control over typhoid, not better control. Of course, the figures we read or have heard about are recorded cases and reported cases. Dr. Doss, I remember, wisely asked whether anybody could tell us what he thinks the percentage of non-reported cases would be relative to the percentage of reported cases. No answer could be given because of many factors.

One factor may be the effect of chloramphenicol. The early, profuse use of chloramphenicol on febrile cases has changed the clinical picture of typhoid or what used to be detected as a typhoid case. There is definitely under-reporting. There are many other reasons, of which we are all aware, which make a real evaluation of the state of frequency of typhoid fever in the U.A.R. very difficult. I hope, as a result of this seminar or as a result of continuous thought stirred by this seminar, we will give enough care to this problem so as to be able to know the magnitude of the problem. Only by knowing the magnitude of the problem will we be able to find ways and means of effective control.

The other significant thing that I observed were some figures which were shown regarding the prevalence amongst

males and females. Statistics can be a double-edged weapon, but the figures gave the impression that typhoid was more prevalent amongst males than amongst females, at a relative percentage of three to two. Of course, we know that one of the main reasons is that females are not as exposed to infection as males, who are running about in the streets most of the time and who eat very often outside their homes. Most females eat only food cooked at home. Maybe this is one of the main reasons, but perhaps another very important reason is that we were hearing or looking at only reported cases. With our habits, especially in rural areas, female patients, even febrile cases might not be reported as easily as male patients, because most of the people know that a reported febrile case will be expected to go to hospital. Many families do not like their mother, wife, or other female to be taken to hospital. So this may be another reason. These two factors alone may be enough to explain the difference from three to two. So, I think one can safely take it that typhoid fever would be as prevalent amongst males in our country as amongst females.

The third question is the difference in prevalence between rural and urban areas. Again, I don't know how significant and reliable the figures shown are. Most probably there is more under-reporting in rural areas than in urban areas. All these different reasons for under-reporting make one more concerned about the status of affairs as regards prevalence of typhoid fever in our country.

Now, when we think or talk about environmental sanitation and the betterment of environmental sanitation, I am not sure of exactly how can we make best use, or enough use, of that for better control of typhoid in the different areas of the country, rural or urban, It is true that we have now much better facilities than before. We should remember that rural areas are now being served by more than two thousand health centers, while 10 years ago we had only 250 centers. We have more than 4000 doctors and a big number of health personnel serving rural areas who did not exist a few years ago. The problem is how to mobilize this army of workers in health

projects with the proper understanding of control methods and of preventive methods? I wish preventive medicine people in executive positions could direct us to ways and means by which we can make better use of the available health personnel now to tackle such a big problem as typhoid fever.

The world of carriers, new or chronic carriers, was mentioned, but it is not yet clear how to control carriers.

I know I haven't said anything new. Maybe I just increased the magnitude of the problem, but I hope as a result of these discussions that we will see better what can be done. I would like to end as I started by thanking NAMRU-3 again and welcoming the honorable doctors who are honoring us with their visit to our country and by their participation in this seminar. I hope such a very high level of scientific seminar for such important health problems will be repeated. Thank you.

DR. FARAG RIZK : Thank you very much, sir. His Excellency has opened many points for discussion, many points for thinking. One of them is the problem of under-reporting. There is another factor I might add, the factor which I might call under-diagnosis. When people get sick, treatment is traditionally prompt, sometimes without making a serious attempt at precise diagnosis. Contributing factors to this may be the deficiency of laboratory facilities and the easy availability of treating medicines and drugs in the community. The floor is now open for discussion on these points.

— Question from the floor regarding definitions of «urban» and «rural».

H.E. THE MINISTER OF HEALTH: Although the question was directed to Dr. Kamal, yet, being the one who used the terms urban and rural, I think I am entitled to explain what I meant. By urban, I mean Cairo, Alexandria, and the capitals of the different Governorates. Otherwise, I meant rural. In our country the population is now about 33% urban and 67% rural.

DR. FARAG RIZK: Dr. Kamal, would you like to comment?

DR. A.M. KAMAL: Mr. Chairman, as the time is rather limited, I didn't expand on points which could be added to what I said. First of all, I want to stress that medical students ought to be educated in environmental sanitation and ought to understand that their collaboration in reporting not only typhoid but other communicable diseases is their first duty. I would estimate that no more than 1% of the recorded cases of typhoid in this country are reported. The others are isolated, or they enter hospitals through other means than referral by general practitioners. If every physician would do his duty of reporting, we could know really what His Excellency the Minister is asking: What is the volume of the problem? I am afraid to say that the volume of the problem is too big. It is more than the recorded cases show. I could multiply by three or five, as far as my experience goes, at least.

About rural and urban areas, the work which has been done by my colleagues and myself has shown that in the villages, the infection is taken during childhood. A lot of gastroenteritis is in childhood, as Dr. Hathout has said. Higgins and Floyd have done much work on salmonellosis and shigellosis during childhood. Five per cent of the children under five years of age with fever in villages around Cairo yielded positive blood cultures for *S. typhi* or *S. paratyphi*. This might be repeated in every village, and the percentage or the ratio might rise in some areas to more than 5%. It is a lot of gastroenteritis. In the environmental conditions of the villages, the population gets sub-clinical infective doses every day through water, flies, and lack of personal hygiene. So, in my own opinion, this is one of the reasons that if you get a fellah from the village, who is a carrier, and you give him a job in the city, in a few months he is negative. This has been proved more than once. He continues to be a positive carrier only because he is repeatedly infected so long as he lives in the village. There are cases where the infecting organism in the same patient has changed from *S. typhi* to *S. paratyphi* or *S. schottmüelleri* B, the same patient being under investigation for weeks or months. So, by rural we mean the village itself.

There is proof that as we rise in urbanization and if we go to the small cities, the small towns, the chief towns of a district and the chief towns of a province, or a governorate, we shall find that the reported cases per hundred thousand rises in the same order. There are a lot of causes for this. In the villages you will rarely find a typical typhoid case. For example, in Calioub area, a demonstration area with the help of WHO, there were 39 medical officers, nurses, and sanitarians working for five consecutive years. There was only one case of typical typhoid during five years. We cannot say the cases were missed, because the houses were visited by the medical officers, nurses, and sanitarians daily, every other day, or every week. So the number of cases of typical enterica in the villages was nearly negative. Why? Because they are getting sub-clinical infections all the time. If we go to the chief town of a district, we find more cases. The ratio rises. If we go to the chief town of the province, again it rises. If we examine the big cities, Alexandria or Cairo, we find a rise too.

Now, Dr. Farag Rizk has said that we are not in the habit of eating out. This is not so in the cities. There is a lot of eating outside. I believe that the number of cases is not connected. If we have so many cases in Cairo, very rarely you will find a connection between one case and another. In volume it is an epidemic. However, if you look epidemiologically at the disease, rarely will you find a relation between cases in Sayeda Zeinab or cases in Ezbekieh. Why? Because they are continuously taking infection. The refuse and the flies might have their effect. Mourad has made an investigation, and he has shown that in schools where itinerant vendors of food stay outside the schools where the students can buy, there is more typhoid and enterica than in those schools where there is a mess hall. So the vendors of food, when one speaks of environmental sanitation must play a role. Food control is an item.

DR. FARAG RIZK: I have a small comment to make. Regarding priorities. I suppose we all agree that there are no priorities in raising the standard of life, we should raise all points together synchronously. If one must put a priority, I

would put it on water supply for many reasons apart from typhoid. However, the standard of life, the social mode of living, the social environmental conditions should all improve together with the health consciousness of the people as Dr. Kamal has said. This is vital, and there has been great achievement in this respect in this country. Conditions have improved tremendously, but there is yet a lot to be done. This is regarding priorities. When we come to urban and rural, there is no sharp line of demarkation between them. It is a matter of gradation, and there is no universal agreement on the definition urban and rural. The definition differs if you are in the U.S., in the U.A.R., or in some country in Europe. Here, we use the administrative definition, and we take the population as a deciding factor. For public health workers, urban and rural means the relative sufficiency of the basic requirements of healthy living. By urbanization we mean that the community has proper water supply in sufficient quantity for human consumption, proper excreta disposal, proper control of vectors, and good communicable disease control. These are the basic requirements of healthful living. If you apply this to our country, we find that villages are strictly rural and that many of our towns are still in the development stage. We cannot call them urban, although administratively they are urban. Capitals of governorates and big cities are really urban, although there is still a lot to be done in them also.

I would like to return to the point of reporting. The deficiency is not only that cases of typhoid are not reported, but it is also that many cases, even those seen by the doctor, are not diagnosed as typhoid. He has no laboratory facilities available to him, and he hurries to a traditional treatment. There has been a study trying to evaluate or to measure the amount of typhoid in a rural community, near Cairo. This experiment was done on the basis of serological surveys and doing statistical studies trying to anticipate the figures. It was interpreted that the amount of infection was about 100 times the amount of disease reported. This does not mean only under-reporting, it may mean, among other factors, the difference between the

word «infection» and the word «disease». «Disease» is a disease entity diagnosed, and «infection» is an experience that leads to certain changes that can be detected by the laboratory.

I agree completely with Dr. Kamal. There is a lot of outside eating in towns, much more than in villages. What I meant is that it is less than in other countries.

2ND PART OF THE SYMPOSIUM

DR. DONALD C. KENT: Your Excellency, I feel that even though we have kept everyone here overtime, it has been worthwhile for all here today. We are taking home with us ideas and thoughts about what we should do when we return to our practices and to our laboratories. However, I feel that we should take advantage of some of our visitors to give us a short critique on where they feel we should go from here in research to better the treatment, control, and our knowledge about the epidemiology of typhoid fever in Cairo and in the U.A.R. Therefore, I would like to ask Dr. Hook, Dr. Hornick, and Dr. Nicolle to please summarize with a short discussion of what they think are the most pertinent things that remain for us to do in this direction.

DR. EDWARD W. HOOK: I shall do little more than list some of the things that have occurred to me during this discussion, We should continue to evaluate new antimicrobial agents. I mentioned a particular combination which seems to be reasonable to evaluate in typhoid fever at the present time. There are probably other things that would be worthwile in this area. One other thing that occurred to me, after hearing the discussion this afternoon, was that more information regarding the etiology of febrile disease as it comes to the physician or in a specific area would be worthwhile. I am sure there are many aspects of this problem, but the specific point I am thinking about relates to the occurrence of typhoid fever in patients who have negative blood cultures or patients who do not have a good response by the Widal test. I think that we might learn something about the additional types of disease that are occurring in the community.

The problem of chronic bacteremia in patients with schistosomiasis is extremely fascinating to me. I think that many aspects of that could receive additional study. One that is of particular interest to me relates to the pathogenesis of this disease and precisely the mechanism of this persistent bacteremia. I do not know if that would help in the control of this particular problem but it perhaps might.

As I mentioned, I think it is important to determine more about immunity in typhoid fever, and I also think it would be of interest to learn something of the role of hypersensitivity in this disease. I obviously would want to maintain a careful vigil for the emergence of resistant strains of *Salmonella* particularly *S. typhi* and the paratyphoid bacilli. It seems to me of very great fundamental importance in evaluating the epidemiology of typhoid and paratyphoid fevers and perhaps *S. typhimurium* to do proper bacterial phage typing of *Salmonella* isolates. This is a matter of considerable importance.

DR. RICHARD B. HORNICK: I must say I think the fine reputation of Egyptians that was transmitted to me before I left the United States has been proven to me today. I wish I was as eloquent as H.E. the Minister and Dr. Kamal. My comments relate to what already has been discussed about public health measures. I too would like to see a broader approach to trying to find resistant strains of *S. typhi* and *S. paratyphi*. I doubt if these strains exist. I would like to have it proven in fact that there are strains that are resistant to chloramphenicol. Furthermore, new drugs should be tried. The disease is here, and there are many cases that are available for study. It was certainly proven in this conference that many well controlled therapeutic trials have been carried out, and I think they should continue. Finally again relating to public health measures and vaccine trials, vaccine has been used effectively in children. In Egypt the disease incidence seems to be greater in children. It may be worthwhile to try the K vaccine or one of the other vaccines in schoolchildren in a double blind trial to see if, under urban or rural environmental circumstances as they exist in this country, the vaccine will or will not be

effective. If it is effective in children, perhaps they should be the group that most attention should be directed toward to try to reduce what appears to be the most common source of typhoid fever in this country. Thank you.

DR. PIERRE NICOLLE: I think it is very important to analyze the frequency with which the various phage types of *S. typhi, S. paratyphi A* and *S. paratyphi B* occur in your country. Of course, it is important to do so in every country, but I think it is particularly important to do so in Egypt. In order to interpret the results of phage typing as applied to one or more epidemic foci, it is essential to know the usual distribution of phage types in the country.

H.E. THE MINISTER OF HEALTH: May I ask two questions of our distinguished guests, and, if there are no answers for them, mention two additional thoughts to take home with us, as you said Dr. Kent. Firstly, we talked a lot about laboratory diagnosis of cases of typhoid, and I want to ask whether other than the classical known methods of laboratory diagnosis, including the phage typing of strains, whether there is or is any hope that there will be in the near future a more easily applicable method of diagnosis for use, for example in rural areas by rural doctors? I have in mind the fact that in diabetes, for instance,the diagnosis of diabetic cases became easy only after the development of methods like the test tape and the sticks. Easily used gadgets are needed which can be used by the patient himself or by the simple doctor in a distant area, methods which do not need sophisticated apparatus or centralized laboratories. Is there any hope that for the diagnosis of typhoid there would be something to test a blood specimen or a urine specimen of a possible typhoid case?

The second question is also very important for us here. A

lot was said today about the relation between chloramphenicol and typhoid, possibly masking some typhoid cases or turning some cases into chronic carriers. In our country the use of chloramphenicol as a drug is absolutely free without any necessary prescription. What is your comment on that? Is it better or not to limit the use of chloramphenicol and make it obtainable only by prescription in a society like ours? What might be the effect of that on diagnosis, treatment, and prognosis of typhoid cases?

DR. EDWARD W. HOOK: Sir, those are very hard questions. I am not sure I can answer them very satisfactorily. As far as the first one is concerned, I don't know of any prospective test that would provide a rapid means of diagnosis in the manner that you outlined. It seems to me, that over the years techniques have been suggested, and in general they have proven ineffective. This is one of the things that I had in mind when I was thinking about knowledge of the cause of febrile diseases in a particular community. Much of our diagnosis, at least as done by physicians, is done on a statistical basis. What is a probability that a certain organism is the cause of a particular syndrome. Of course, this varies in different areas. Now that is not to say that we would not want to apply the best diagnostic techniques that we have available, but the point is that when we have to initiate therapy, most of the times the diagnosis is a presumptive one. Many times it is based only on the clinical picture. So, perhaps additional information about the occurrence of febrile disease would be of value. As far as the other point is concerned, I am not sure that I know enough about how chloramphenicol is distributed in Egypt to answer the question accurately or well. However, it would seem to me that in areas where typhoid is highly endemic, the chances of the clinical diagnosis being relatively accurate are good. It

would be my guess that use of chloramphenicol probably would be doing far more good than it would harm. At least this would be my impression. This is not to say that we should not aim towards making our therapy as specific as possible, but, I think if these opportunities for diagnosis are not available, then one has to rely on the basis of the clinical picture and do what seems best. In most of the cases we have been talking about, this would be the administration of chloramphenicol.

DR. DONALD C. KENT: I wish to thank everyone for coming, for giving us their attention, and for giving us their thoughts. I hope that in a not too distant future we can all be together again on another subject similar to these last two: Meningitis and typhoid fever. Thank you very much.

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IMMUNOLOGICAL CONTROL OF TYPHOID FEVER

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The investigations presented in the previous paper in this symposium described the human model of typhoid fever. Under the circumstances of these studies it has been possible to control the size of infecting dose as well as the phage type of organism. Vaccines can be evaluated in such a model by challenging vaccinated volunteers with various doses of typhoid bacilli. These results would quantitate vaccine efficacy. It is the purpose of this discussion to present evidence pertaining to immunological control of typhoid fever.

Evaluation of Baseline Titers and Subsequent Disease:

Availability of volunteers prior to time of induction of typhoid fever enables estimation of baseline serological experience to the disease. In the volunteers involved in the studies designed to develop the dose response curve for *S. typhi,* baseline serum antibody titers (O, H, and Vi) were tabulated. No correlation could be drawn between the absence or elevation of any antibody

titer and subsequent clinical course. The statement held for each challenge dose employed. For example, in the group receiving 100,000 organisms, just as many volunteers lacking antibodies became ill as those who had significant levels of O, H, or Vi antibodies.

These antibodies are known to enhance serumcidial activity especially O and Vi antibodies. This reaction requires complement levels increased early in the course of typhoid fever. Whether this increase was associated with increased serumcidial activity was not determined. It is intriguing to speculate that during this phase — early bacteremic — the host has attempted to confine the infection through enhanced bacterial activity of the serum. Unfortunately, when baseline serumcidial activity was compared to subsequent clinical disease, no correlation could be made. Enhanced activity did not protect the volunteers from disease to any greater degree than those men with low or absent titers. *Relapses :* Relapses appear to represent a lack of immunity to *S. typhi.* They are common in typhoid patients, occurring in about — 8 to 10% of patients in the pre antibiotic era and 15-20% in those treated with chloramphenicol. These data imply that antibiotic therapy interferes either with the development of «protective antibodies» and/or cellular immunity. The mechanisms of action of chloramphenicol would support this thesis since protein synthesis is inhibited through the blockade of messenger RNA. Indeed, studies have been reported indicating impairment of antibody development in man by chloramphenicol. If a protective antibody is inhibited, the nature of such inhibited antibody in antibiotic treated patients is unknown. There was no correlation between humoral O, H or Vi antibodies and tendency to relapse. Relapses occurred at the peak of circulating antibody titers, and about 15 days following cessation of antibiotic therapy. Recrudescence appeared to occur after persisting typhoid bacilli have multiplied sufficiently to cause overt disease. Relapses usually were mild and sometimes self-limiting, which implies that the host is better able to eliminate the offending organism, presumably through stimulated macrophages or other cell associated inhibitory mechanisms.

Reinfection Typhoid:

Reinfection with typhoid fever is rare. In 1953, Marmion[1] reported two epidemics of typhoid fever occurring within 5 months in a British Air Force camp. Fifty-four men who had developed typhoid fever in the first epidemic were reexposed in the second. The subsequent attack rate in this group was 20% compared to an overall rate of 34%. These data suggested that natural disease conferred moderate immunity. However, a portion of this incomplete immunity might be explained by different phage types involved in the two epidemics and probably a larger dose was ingested in the second exposure. In the present study, volunteers were selected because of previous induced disease and rechallenged with identical doses of the same phage type organisms, two to twelve months later. Their illness rate was now about 25%. In this group of initially susceptible volunteers, perhaps significant immunity was induced. No correlation between resistance and O, H and Vi antibody levels was demonstrated.

Typhoid Carriers: A striking example of intestinal resistance to infection with *S. typhi* is the typhoid carrier. These people serve as the reservoir of typhoid fever. There may be as many as 10^{11} virulent typhoid organisms per gram of feces. These organisms reside in scarred foci of the biliary tree, migrate through the bile ducts and over the vast surface area of intestinal epithelium yet they do not cause typhoid fever in the carrier. Frequently, antibody levels are not elevated and in a few carriers tested, tolerance to endotoxin is not apparent. Thus, these virulent organisms are excluded from the host presumably by local humoral or cellular immune mechanisms. An improved understanding of this remarkable example of microbial persistence and the associated local defenses may provide leads to better control measures against enteric infections. One interesting and as yet unconfirmed observation was recently reported by Chernokhovostova and colleagues.[2] They noted that typhoid carriers were deficient in IgM and did not respond to Vi antigen administration with any IgM type antibodies. This information suggests that carriers are selected because of their deficiency of IgM which is very active

in promoting serumcidal activity. The obvious anatomical alterations in the biliary tract are very important in the production of the carrier state. A deficiency of IgM, and impaired drainage because of scarring of the biliary tree would produce an environment conducive to prolonged bacterial persistence.

Vaccine Field Trial Results: Killed parenterally administered vaccines are known to boost bacteriocidal activity of serum through the production of O, H and Vi antibodies. As noted the enhanced killing power of serum could not be correlated to subsequent clinical illness. Cellular hypersensitivity may occur, but its role as a defense mechanism in human typhoid illness needs elucidation. The purpose of the volunteer studies has been to determine whether typhoid vaccines can prevent or ameliorate the clinical illness. The evidence presented thus far has deliberately attempted to minimize the importance of known vaccine effects in defense against typhoid. Immunoprophylaxis has been difficult to document. The most convincing field trials designed to evaluate typhoid vaccines were initiated by the World Health Organization in 1955 and continued for 12 years.[3,4,5] These trials were well controlled and included the testing of three vaccines. Two test vaccines, K and L were prepared from the standard Ty 2V strain at the Walter Reed Army Institute of Research. K vaccine was monovalent and contained acetone inactivated and dried typhoid bacilli which had retained Vi antigen. Vaccine L consisted of the conventional heat killed formalin preserved typhoid bacilli. This treatment destroyed most of the Vi antigenicity. The control vaccine was tetanus toxoid. Studies were conducted in Yugoslavia, Poland and British Guyana using identical vaccines. Similar studies were also conducted in Russia.[6] Children and adults were participants in the evaluation and were recruited as volunteers. The best results were obtained in children. K vaccine appeared to be superior to L in inducing protection against typhoid fever. After seven years in Guyana there were 146, 16 and 49 confirmed cases of typhoid fever in children in the placebo, vaccines K and L groups respectively. An unexpected result of this trial was the finding that only one (vaccine K) and four (vaccine L) cases of typhoid fever occurred in a total

of 10,000 children given only one dose of vaccine. Twenty-two cases occurred in the control group. Ashcroft[7] suggests that in Guyana and other endemic areas, repeated ingestion of subinfective doses of typhoid or related organisms may result in an immunized population and vaccine will enhance the protection. These results confirm that typhoid vaccine lowers the incidence of disease in susceptible children in endemic areas. The means by which this is accomplished is unknown. The results in adults were somewhat confusing. Different rates of protection was obtained in various countries. Also vaccine L appeared to be less effective than K in inducing protection in adults. Conceivably, an increased level of background immunity was present as compared to children and yet the vaccines apparently induced less resistance. In all countries the vaccine-induced immunity was less in adults than children. Perhaps, adults had an increased opportunity to ingest larger doses of organisms.

Vaccines K and L, were appraised in Pristina, Yugoslavia, an area of high typhoid endemicity resulting from ingestion of heavily contaminated water.[5] Those persons not volunteering for the typhoid vaccine study showed six times the attack rate of the tetanus toxoid control group. These data suggest that highly motivated persons who volunteer for studies are concerned with all preventive measures important in avoiding typhoid fever. They would, therefore, be less likely to ingest large numbers of enteric pathogens.

Vaccine Evaluation in Volunteers: Evaluation of these same vaccines was conducted in volunteers.[8,9] In this experimental situation, control of the infectious dose, strain involved and knowledge of humoral antibodies allowed for quantitative and qualitative analyses. In addition to the K and L vaccines, Vi antigen provided by Drs. Maurice Landy and Joseph Lowenthal was also employed. Vaccines K and L were given in three 0.5 ml doses, the first two at weekly intervals and the third one month after the second dose. Single 50 ugm doses of Vi antigen were given subcutaneously.

No protection was afforded to volunteers challenged with an

ID_{50} dose (10,000,000) of *S. typhi* or higher (Table 1). Illness occurred in vaccinated and unvaccinated subjects in spite of high antibody titers prior to challenge. Indeed, some vaccinees became ill sooner than their nonimmunized controls. When clinically apparent typhoid infections developed following ingestion of either low or high infectious doses, the severity of illnesses were similar.

TABLE 1 — Typhoid Fever: Incidence in Vaccinated Volunteers Following Graded Challenges

Vaccine	Dose of *S. typhi* Administered					
	10^9		10^7		10^5	
K	2/3*	67%	12/28	43%	4/43	9%**
L	3/4	75%	13/24	54%	3/45	7%**
Vi	6/7	86%	10/14	71%	3/17	18%
None	4/4	100%	15/30	50%	28/104	24%

* Each fraction represents number with disease over number challenged with *S. typhi* — Quailes strain.

Effectiveness of killed vaccines = 100 (b-a)/b; a = incidence in vaccinated group, b = incidence rate in controls. See text.

** Chi square test — K versus control $p < 0.05$
L versus control $p < 0.02$

all vaccines including Vi, $p < 0.01$.

At the ID_{50} (100,000) challenge dose, protection was demonstrated between vaccinees and controls. Only about 9 per cent of the vaccinees (K and L) became ill, in contrast to 27 per cent of the non-vaccinated controls. The currently available vaccines thus showed effectiveness of 67 per cent against an infecting dose of 100,000 bacilli. Attempts to correlate prechallenge levels of agglutinins to O and H antigens, of Vi hemagglutinins and serumoidal activity with subsequent clinical course were made. No direct relationship was found. Immunoglobulin analyses were

carried out on a number of the volunteers sera. No grouping of titers of IgG and IgM and subsequent response to virulent organisms was apparent. Thus analysis of humoral antibodies failed to show any demonstrable effect in protecting volunteers from illness.

Seventeen volunteers given a single dose of purified Vi vaccine showed definitely less protection than vaccines K and L. Only a single injection was given and perhaps increases in concentration and/or frequency of administration could improve the results.

These studies showed a correlation with the WHO field trials and suggested that the infecting dose of typhoid bacilli in nature approximates 100,000 organisms which might result from a water-borne exposure. Vaccine-induced resistance would falter when contaminated foods, which upon prolonged incubation would contain huge numbers of organisms per gram are ingested.

ORAL VACCINE TRIALS

Little is known of the effect of parenterally administered vaccine in stimulating specific local antibody in the intestine. It is in this organ that the initial contact of the pathogenic organisms and host occurs. Vaccine stimulated resistance most likely affects the progress of infection only after the primary defenses of the intestinal wall have been broached. For this reason it was decided to attempt to stimulate intestinal barriers. Several potential oral vaccines have been available for evaluation. Killed bacteria in the form of keratinized tablets are uniformly employed as vaccines in various European countries. One of these was tested for immunogenicity and efficacy. Taboral (Swiss Serum Vaccine Institute), was a monovalent preparation consisting of 100 X 10^{9} acetone-killed *S. typhi* (strain Ty2) per tablet.

Four fold rises of serum agglutinins did not consistently develop in volunteers given prescribed doses of Taboral (six tablets per person over 3 days). When 12 tablets of Taboral were given to 29 volunteers (twice the recommended dose), rises of somatic (O) agglutinins, flagellar (H) agglutinins and Vi antibodies occurred in 20, 25 and 50 percent, respectively. There were no intestinal or systemic reactions to the vaccine.

Volunteers who ingested 12 tablets of Taboral were given approximately 100,000 virulent *S. typhi* of the test strain. Clinically detectable typhoid fever developed in 38 per cent of the vaccinated and 54 per cent of the controls. Positive stool isolation of *S. typhi* was noted in 33 per cent of those vaccinated and 63 per cent of the control volunteers. The number of vaccinees was small, a total of 21. Yet, the results suggested that there was a diminution of multiplication of typhoid bacilli in the intestinal tract following oral vaccination with large doses of killed organisms. There was no correlation with respect to the serum agglutinin titers and clinical response to infection in the vaccine group.

Six tablets of Taboral gave no protection when such vaccinees were given 100,000 pathogenic typhoid bacilli. Attack rates approximating 40 per cent were noted in vaccinees and non-vaccinated controls.

These killed vaccine preparations did not appear to induce a level of immunity comparable to that following vaccine given parenterally. However, the incidence of illness in control volunteers in these oral vaccine trials were higher than observed in earlier studies. Perhaps a lesser challenge would have demonstrated a more marked beneficial effect. The finding that smaller numbers of volunteers showed positive stool cultures in the large dose Taboral group suggested that local immunity may have developed consequent to the massive antigenic stimulus.

The above investigations plus information obtained from evaluations of living shigella vaccines, suggested that repeated doses of an attenuated strain of *S. typhi* might lead to effective resistance at the portal of entry. Reitman[10] selected, by antibiotic stress, a streptomycin dependent mutant strain, S-27, of *S. typhi* which was an effective vaccine in mice. Large doses (10^9) of this strain were ingested by volunteers simultaneously with streptomycin given orally. Multiplication was confirmed by repeated isolation of S-27 strain from stools (using SM containing media) for the seven days that streptomycin was given. Other volunteers given similar numbers of organisms without streptomycin had no fecal cultures positive for strain S-27. This strain

contains Vi antigen, but the one step mutation creating streptomycin dependency led to complete avirulence. No evidence was found that intestinal penetration occurred. Blood cultures and serological studies were negative. Challenge of these vaccinees with an ID_{50} dose of the virulent Quailes strain resulted in an attack rate comparable to controls. Subsequent studies have employed multiple (4-6) huge doses (10^{11}) of S-27 administered concurrently with streptomycin and sodium bicarbonate. No reactions to this combination have been noted, further attesting to the lack of human virulence of S-27. Evidence of humoral antibody stimulation was confirmed in some of the men. Four fold rises in H, O and Vi antibodies were found in 42, 6 and 9%, respectively.

Preliminary challenge studies have been conducted in 30 of these orally immunized volunteers and evidence of protection from disease has been obtained. In addition, a very striking difference was noted in the number of positive stool cultures obtained in the vaccinated versus the control population. There was a significant reduction in the vaccinees, with most resistant individuals having a single or at most two positive cultures during a six week followup period. Most controls had repeatedly positive stool cultures. Thus, it appears that this attenuated strain can enhance local immunity of the intestinal tract and effctively prevent typhoid bacilli from entering a site in which to multiply. Perhaps now we have acclimatized the unsophisticated intestinal tract to typhoid fever through the use of multiple exposures to an attenuated strain. Additional studies are needed to confirm this finding. It is conceivable that similar approaches could be utilized to prevent other bacterial enteric diseases.

Conclusions: The parenteral administered typhoid vaccines confer limited protection to population groups at risk. This is a quantitative immunity which can be overcome by large numbers of ingested *S. typhi*. Recent preliminary studies have indicated that the gastrointestinal tract may be immunized so as to prevent the early invasion of *S. typhi* into the host from the gut. This appears to be a promising area for further research.

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We are most grateful for the past and continued cooperation of the volunteers who have made these studies possible. The officials of the Maryland House of Correction have been most helpful in assisting us with these studies. Many physicians associated with the Division of Infectious Diseases have been instrumental in carrying out work described in these papers. Their names have been published elsewhere and this review is dedicated to their help.

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PATHOGENESIS OF TYPHOID FEVER

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The induction of disease by an infectious agent is dependent upon many factors. A formula depicting the relationship of the most important of these is as follows:

$$\text{Infectious Diseases} = \frac{\text{Number of bacteria X Virulence}}{\text{Resistance of Host}} \text{ X K}$$

Actual determination of the number or virulence of a bacterial species responsible for naturally occurring disease in man is virtually impossible. The number of organisms actually ingested; for example *Salmonella typhi,* is conjecture. Data pertaining to this point are acquired after the incubation period has passed and the first few cases of disease have occurred. Human virulence of pathogenic organisms is only relative and can rarely be predicted from naturally occurring disease. The resistance of man to an infectious agent is also difficult to document because

of the important influences of numbers of organisms, virulence, previous exposure and non specific defenses. Thus in an attempt to evaluate the efficacy of a vaccine in a field trial many unknown factors are involved. Partly for the reasons mentioned and in addition because of the lack of a suitable experimental animal, typhoid fever has been induced in volunteers in order to quantitate the efficacy of typhoid vaccines. With this investigative approach many of the variables mentioned above can be controlled. It is the purpose of this presentation to discuss some of the pathophysiological alterations observed in the volunteers. The subsequent paper discusses the relationship of these to the control of typhoid fever.

Susceptibility to Typhoid Fever: Susceptibility to typhoid fever may be conditioned by the nature of the environment in which man lives. In many areas of the world today (for instance Chile and Egypt), the highest incidence of typhoid fever is found in children. In these locales, it may be assumed that the first few exposures to typhoid organisms usually result in infection and a few persons develop disease. Adults on the other hand, having had frequent encounters with these microorganisms (mild self-limiting infections), have developed significant resistance. Other countries present less opportunity to acquire repeated subclinical infections and have populations who are susceptible at all ages. Thus, in the U.S., scattered epidemics occur following food contamination by a carrier and all exposed are at risk. This hypothesis has been promulgated by several authorities.[1,2,3]

In the studies reported below, the population studied presumably represents a highly susceptible group, not comparable to adults residing in other areas of the world where typhoid is more common. The volunteers have an unsophisticated intestinal tract in terms of experience with typhoid bacilli. Their reactions to various numbers and strains of typhoid organisms would be representative of those of persons residing in countries with a low incidence of typhoid fever. However, the results obtained in these men in terms of susceptibility to infection and levels of induced immunity may have added meaning because of this lack of previously acquired resistance to typhoid. Vaccine trials conduc-

ted in endemic areas measure the additive effect of vaccine immunoprophylaxis upon the background level of acquired resistance. Under these circumstances, such studies might be expected to provide various results depending on the extent of this background immunity.

The administration of known numbers of a well characterized bacterial strain to individuals with a known vaccine history allows for precise determinations of acquired resistance. Unfortunately, in nature, the virulence and number of ingested organisms are unknown, and in a specific person the state of resistance at the time of exposure cannot be determined. Volunteers were selected only after they were identified as healthy and thus could be expected to have those general nonspecific attributes which aid resistance to disease. It is in such healthy, informed volunteers that information regarding pathogenesis and resistance to typhoid fever has been gathered.

NATURE OF INDUCED DISEASE

Clinical Description : A pathogenic strain of *S. typhi* (Quailes strain) obtained from a carrier, was used to induce infection; the exact number of viable organisms required to infect, their antigenic content, mouse pathogenicity and time of infection were known. This strain, which possesses significant amounts of the Vi (envelope) antigen, was propagated on solid and liquid media and harvested approximately 6 hours after 37°C incubation. It was suspended in an ounce of milk and administered by gargling and swallowing. The characteristics of this strain have been well studied and its virulence relative to other classic typhoid strains has been defined. This information is presented in a subsequent section.

The symptoms and signs associated with the illness resulting in volunteers were identical to those observed in patients with naturally acquired typhoid fever. Fever was the earliest indication of disease, rising over a 2-3 day period in a step-wise fashion. Headache and abdominal pain occurred shortly thereafter. Tenderness to palpation in the lower abdominal quadrants with as-

sociated sensation of displacing, under the palpating fingers, loops of bowel filled with air and fluid, was a pathognomonic physical sign. Subsequently, anorexia, myalgia and fatigue occurred. Chills and sweats were uncommon; the latter appeared only after antibiotic therapy was started. Herpes simplex infection was not observed. Chloramphenicol treatment always aborted illness.

Once illness occurred, the clinical courses were comparable regardless of the dose of the infectious inoculum. The median incubation period varied inversely with the size of inoculum. However, within each group incubation periods varied greatly (see Table 1).

TABLE 1

Relationship of Dosage of *S. typhosa* — Quailes Strain to Disease

Number of Viable *S. typhosa*	Volunteers with Disease		Incubation Periods. Days	
	Total Challenged	Per Cent	Median	Range
10^9	40/42	95	5	3—32
10^8	8/9	89		
10^7	16/32	50	7.5	4—66
10^5	32/116	28	9	6—33
10^3	0/14	0		

Clinical manifestations were arrested by chloramphenicol administration, usually the volunteers were afebrile by the third to fifth day of therapy. A standard protocol was established in order to estimate vaccine effect. Thus, antibiotic treatment was begun when the oral temperature reached a level of 103°F, or greater and persisted for 24-36 hours. The majority of patients developing disease reacted in this rather uniform fashion. A minority were treated when their temperature remained under 103°F

for longer than 5 days and their clinical condition warranted therapy. During the course of these studies, 250 men received antibiotic treatment (215 with chloramphenicol) for typhoid fever. There were two instances of moderate hemolytic anemia which occurred before chloramphenicol was given. Several volunteers developed temporary episodes of confusion. There have been no permanent typhoid carriers.

Dose Response Data: The development of typhoid in man following various doses of bacilli was determined in order to establish a meaningful challenge dose for immunized volunteers. Attack rates for disease were determined when the illness consisted of toxic symptoms and signs such as headache, malaise, anorexia and an oral temperature of 103°F or greater for at least twenty-four to thirty-six hours. At this point specific treatment was initiated. The infectious dose (ID) of *S. typhi* (Quailes strain) for healthy volunteers is shown in Table 1. The ID_{50} is about 10^7 (10,000,000) bacilli as compared with a rate of approximately 95 per cent in volunteers ingesting 10^9 organisms. The most commonly employed dose, 100,000 *S. typhi,* caused disease in 28% of the volunteers, whereas the smallest dose employed, 1000 cells, failed to induce disease in any of the 14 volunteers. Scant information is available to compare these results with numbers of organisms causing naturally acquired disease. Most data have been acquired after the incubation period was passed and the first few cases of disease have occurred. The number of organisms actually ingested in contaminated water or food is conjecture. If the incubation periods are well documented, as in the Zermatt epidemic of 1963[4] it is possible to compare attack rates in the naturally acquired cases with our present volunteer data and thereby estimate the dose of organisms involved. Indeed in Zermatt, it appears that certain groups of tourists, i.e. those with well documented incubation periods, probably ingested less than 100,000 typhoid bacilli. This inference seems justified, since the virulence of one strain isolated from patients in the epidemic has virulence comparable to the Quailes strain.

Characterization of Virulence Factors in S. typhi: In addition to the numbers of organisms ingested, the antigenic makeup

is another determinant in the virulence potential of a particular strain. Sorting the pathogenic role of each antigen in causing disease delineates the factors that need to be inhibited in order to prevent disease. This information could lead to better vaccines. The data cited above for the infectious dose of *S. typhi* for man was obtained with one strain which contained Vi antigen. It caused typhoid fever and was isolated from a carrier. Other strains with differing antigenic makeup were studied for their human pathogenicity, Table 2. The Zermatt strain obtained from Dr. Vischer of Basal, Switzerland, is one of the strains isolated during the epidemic occurring in Zermatt in 1963. This strain contains Vi antigen and is highly virulent for mice by intra-peritoneal injection. Vaccines have been derived from the classic Ty2V strain, isolated by Felix from a patient in a small Russian village in 1915[5]. A strain which lacked Vi antigen was derived from Ty2V cultures and called Ty2W.[5] Absence of Vi antigen renders this strain less pathogenic for mice. Strain 0-901 which was obtained by Felix[5] in the same locale as Ty2V lacks both Vi and H antigens. Mouse virulence is of the same order for the 0-901 as Ty2W; both significantly less than Ty2V. These strains differed not only in their antigenic makeup but had an additional variable of being long established laboratory cultures, far removed from viable human tissue.

Following a dose of 10,000,000 organisms, disease rates were significantly higher in volunteers who ingested Vi containing strains than with non Vi strains. 51% versus 25% — P value = <.05. Vi antigen thus appeared to be an important determinant of human virulence for typhoid bacilli. This was thought to be a non-toxic antigen. Its virulence enhancing effect in animals related to its role as an envelope antigen which protects «O» antigen from «O» antisera. In this manner it interfered with serumcidal activity. Also Vi antigen may be a deterrent to phagocytosis. Similar roles in man may be inferred from the data in these studies.

These non-Vi organisms were difficult to isolate from blood and fecal specimens. Attempts to isolate typhoid bacilli were successful in 40% from the blood or feces in contrast to over

TABLE 2

Virulence of Certain Strains of Typhoid Bacilli for Man. Effect of Vi Antigen on Virulence
Challenge Dose Approximately 1 X 10^5 Organisms

Strain	LD_{50} mouse Virulence (IP)*	Human Virulence		
		Disease[1]	Infection[2]	No Infection[3]
Quailes	2.6 X 10^7	16/30	12/30	2/30
Zermatt	3.0 X 10^4	6/11	4/11	1/11
Ty2V	3.0 X 10^6	2/6	3/6	1/6
	Totals (Vi Strains)	24/47 (51%)**	19/47 (40%)	4/47 (9%)
0-901	3.11 X 10^8	6/20	6/20	8/20
Ty2W	1.9 X 10^8	4/19	10/19	5/19
	Totals (Non-Vi Strains)	10/39 (26%)**	16/39 (41%)	13/39 (33%)

* Mouse virulence evaluated by IP inoculation of organisms in saline. Zermatt strain virulence was determined with organisms in gastric mucin.

** Chi square test difference between incidence of disease caused by Vi and non Vi strains is significant, $P = < 0.05$

1 Disease = Temperature of 103° or greater for over 36 hours and treatment with antibiotic.

2 Infection = Low grade fever or significant serological response, or positive blood culture or excretion of *S. typhi* in stools for more than 5 days and no specific therapy.

3 No infection = No clinical, cultural or serological evidence.

80% of volunteers infected with fully virulent strains (Vi antigen containing). The clinical illness of these volunteers infected with non-Vi strains was typical of enteric fever, however, host ability to readily phagocytize such strains probably accounted for fewer positive cultures. Serological data on the other hand, was of more help in confirming these cases. Conceivably, individuals infected naturally with similar less virulent strains may present as patients with obscure fever and negative blood and fecal cultures.

Interaction of S. typhi and the Gastrointestinal Tract : The rapidity and site of penetration by typhoid bacilli of the intestinal epithelial lining in man is unknown. In volunteers it was possible to rule out the pharynx as a significant portal of entry by having participants expectorate the gargled suspension (10^9). In no instance was disease initiated.

The human stomach with its marked acidity is regarded generally as a nonspecific host defense for degradation of swallowed bacteria. Attempts to isolate *S. typhi* from the gastric secretions of volunteers were successful for as long as 30 minutes after ingestion. This organ may play a significant role in defense against enteric infections since the number of organisms are reduced through its inhibitory effect. Small doses of sodium bicarbonate given to volunteers prior to ingestion of shigellae has significantly enhanced the rates of illness and stool isolates. Gastritis is a consequence of shigella infection in monkeys and in man, suggesting that these organisms can penetrate the gastric mucosa. However, such a condition in typhoid is rare. Specimens of jejunum obtained by biopsy after infection with *S. typhi* have shown inflammatory changes.[6] These findings imply that salmonellae entered the mucosa at these sites. These biopsy specimens were obtained late in the incubation period, prior to demonstrable bacteremia. Conceivably, typhoid bacilli were limited to the draining lymph nodes at this time.

Additional information pertaining to the rapidity with which typhoid bacilli enter cells or find other areas in which to survive hostile environmental factors can be inferred from re-

sults of studies designed to test the efficacy of antibiotics to prevent typhoid fever. Four volunteers were given an ID_{95} dose of typhoid bacilli; 24 hours later each was begun on chloramphenicol therapy in doses of 1.0 gram three times a day. Two of the men continued this regimen for 7 days and the others for 28 days. None developed illness while on therapy. Blood was cultured during the late stages of the expected incubation period and continued periodically thereafter. Despite administration of this effective drug, positive blood cultures —were obtained in one of the four and all showed significant increases of antibody titers while receiving the antibiotic treatment; simultaneously, control subjects were ill and demonstrated similar laboratory findings. One of the two volunteers treated for 7 days chemoprophylactically subsequently developed disease about 9 days after the drug was stopped. Those men maintained on antibiotic for 28 days failed to develop symptoms or signs of illness but did show antibody responses. These data suggest that infecting organisms found an intra-cellular habitat within 24 hours and therefore were not eliminated by the antibiotic. Tissue culture studies performed by Showacre et al[7] demonstrated intracellular persistance of typhoid bacilli despite the presence of antibiotic in the menstruum, i.e. «antibiotic indifference»; when the antibiotic was washed out (after 21 days), the organisms multiplied.

Typhoid bacilli are susceptible *in vitro* to many antibiotics. Several drugs are markedly bactericidal, e.g., polymyxin, colymyxin, colymycin, yet none cures patients with typhoid fever.[8] Antibiotic control requires a drug like chloramphenicol that perhaps inhibits the growth of the pathogen by effectively altering both cellular and bacterial metabolism through interference with nucleic acid synthesis. The process is slow; patients require 3 1 2 to 5 days to become afebrile and irradication is incomplete. Stool cultures remain positive for several weeks to months following therapy and relapses are common (15-20%). The unique response of *S. typhi* to antibiotic *in vivo*, i.e. only to chloramphenicol (somewhat less to ampicillin) attests to the effective adaptive capabilities of this organism in man. A means of aborting these capabilities would provide an excellent method of control.

Japanese and American investigators[9,10] showed that streptomycin inhibits growth of anaerobes of the genus Bacteroides and of lactobacilli, each partially responsible for maintaining an acid pH in the intestine of mice through their production of short chain fatty acids. In the acid medium, salmonellae were inhibited. Thus, in the mouse antibiotic given orally lowered the dose needed to induce disease from 10^6 to less than 10 *S. enteritidis* organisms.

Oral pre-challenge of volunteers with streptomycin (no effect on the typhoid bacilli) may have similarly influenced intestinal defense mechanisms. Whereas clinically detectable typhoid fever did not occur in 14 volunteers given 1,000 viable typhoid bacilli, one of four treated volunteers became clinically ill. Concentrations of short chain fatty acids change during some enteric diseases but their role in protecting the large intestine from colonization is not yet defined.

DISCUSSION

The evidence presented in this paper has emphasized the interaction of the typhoid bacilli and the gastrointestinal tract. This is the organ system which ultimately decides the course of the typhoid infection — the early stages of pathogenesis are accomplished at this level. Large numbers of ingested typhoid bacilli can overcome specific and non-specific defense mechanisms in the gut. Smaller numbers may induce disease if certain host factors fail to contain the initial surge of the pathogens. Many factors in addition to those mentioned are involved in the outcome of this encounter. A better understanding of these can lead to improved means of control. The brief mention of bacterial interference manifested by inhibition of salmonella by short chain fatty acids is but one mechanism that can influence pathogen survival. Antibiotics produced by bacteria i.e. bacteriocinslike

colicins from *E. coli*, may be important in preventing pathogens from establishing intraintestinal multiplication. Metabolic interference can be demonstrated *in vitro* and hence may be significantly involved when ingested enteric pathogens encounter species of the nosocomial flora. Motility of the intestinal tract is an important factor influencing shigella and salmonella infections in animals. Guinea pigs can only be infected with shigella if motility of the GI tract is slowed with paragoric. These few examples illustrate the complexity of pathogen-gastrointestinal interaction; most of these are of unknown importance in man. Yet this type of information would be of interest in order to enhance specific and non specific resistance to enteric infections. Conventional typhoid vaccines have been only of minor significance in preventing disease and probably have no effect in stimulating resistance at the portal of entry. The continued prevalence of typhoid fever throughout the world enforces the need of improving means of control.

Little has been mentioned in this presentation of the role of endotoxin in producing signs and symptoms of typhoid fever. It has been purposefully avoided because of the complex nature of the reactions associated with endotoxin. However, a few statements are needed to indicate those areas where some data has accumulated from the volunteer studies and which bears on these presentations. Since this substance is a powerful fever producing agent and is contained in the cell wall of the typhoid bacillus, it is reasonable to assume it is involved in production of the prolonged fever of typhoid disease. However, this is not simply a result of continuous release of endotoxin into the circulation. Instead a state of hypersensitivity develops in man and the concentration of endotoxin in local lesions, not necessarily that circulating in the blood stream, is probably the cause of the fever. The mechanisms responsible involve stimulation of sensitized

lymphocytes and macrophages in the typhoid lesions. These cells release the intermediary compounds causing fever such as endogenous pyrogen which react with temperature regulating cells in the hypothalmic area. Circulating endotoxin is rapidly removed from the blood stream by elements of the reticuloendothelial system in the liver. The clearance is accelerated when one becomes tolerant to endotoxin a condition acquired during typhoid fever. Antibodies to endotoxin are involved in the development of tolerance. These antibodies however cannot be correlated to immunity. Measurement of cellular immunity induced by endotoxin remains a crucial determinent of the significance of endotoxin in the defense against typhoid fever. Obviously, the understanding of endotoxin's participation in the genesis and control of typhoid fever has not yet reached a level of comprehension which would allow consideration of it as a means of practical control.

The development of a model of typhoid fever in man has allowed for the quantitative analysis of vaccines. Results of these studies are presented in the second paper.

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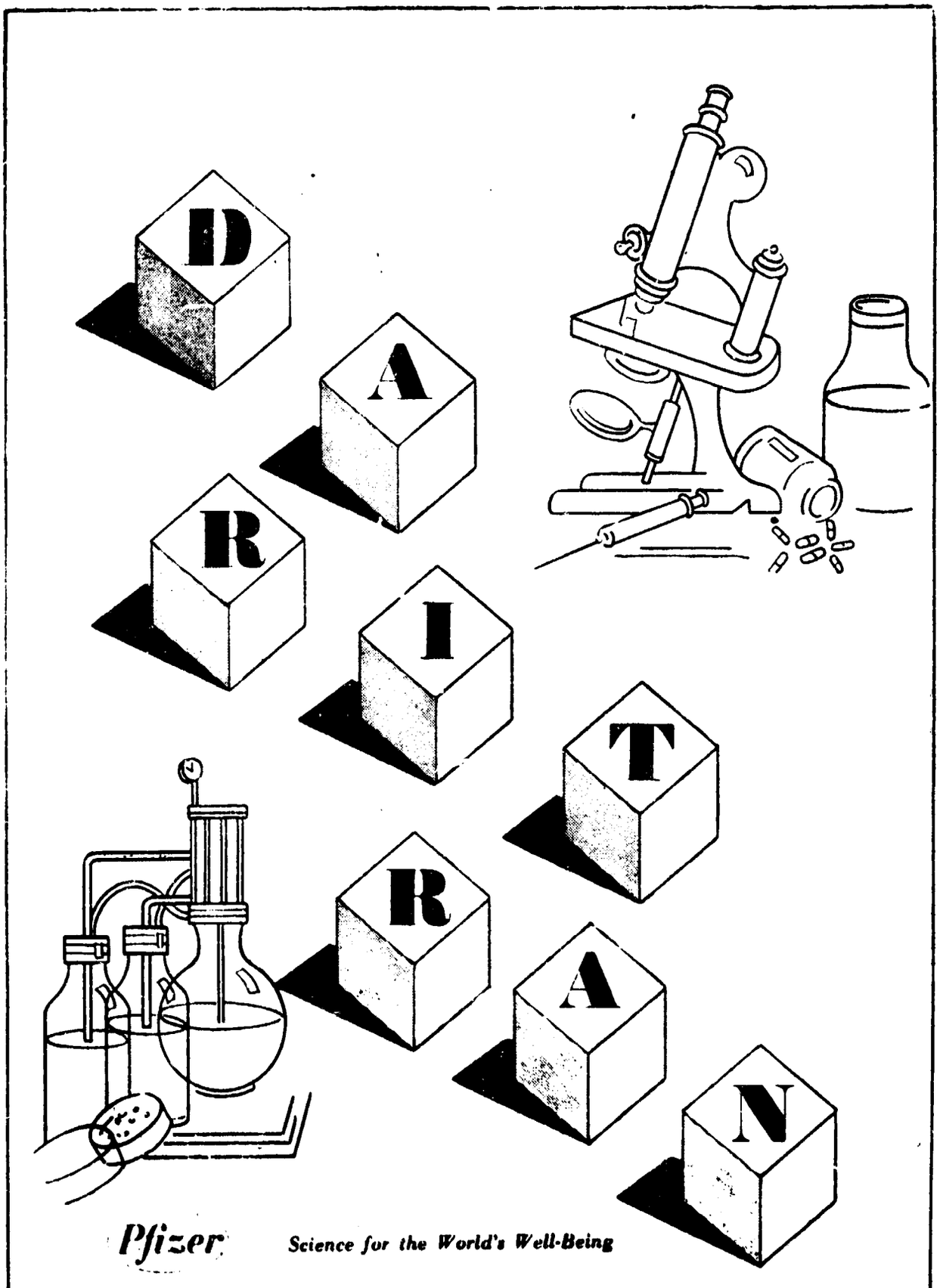

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